STUDY OF ANTIHYPERLIPIDAEMIC ACTIVITY OF *ALSTONIA SCHOLARIS* LEAVES

The Tamil Nadu Dr.M.G.R Medical University Chennai

In partial fulfillment of the degree of

MASTER OF PHARMACY

(Pharmacology)

Submitted by

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Under the guidance of

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This is to certify that this dissertation entitled "**STUDY OF ANTIHYPERLIPIDAEMIC ACTIVITY OF *ALSTONIA SCHOLARIS LEAVES***" Submitted by **Mr.MUHAMMED THWAYYIB.P.K** to The Tamil Nadu Dr.M.G.R Medical University, Chennai in partial fulfilment for the degree of **MASTER OF PHARMACY IN PHARMACOLOGY** is a bonafied work carried out by the candidate under the guidance and supervision of **Dr. C. SENTHIL KUMAR M.Pharm., Ph.D** Associate Professor in the department of pharmacology, Karpagam College of Pharmacy Coimbatore-32.

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Date: Co-guide

DECLARATION

I hereby declare that this dissertation entitled **"STUDY OF ANTIHYPERLIPIDAEMIC ACTIVITY OF *ALSTONIA SCHOLARIS* LEAVES "** submitted by me, in partial fulfilment of the requirements for the degree of **MASTER OF PHARMACY IN PHARMACOLOGY** to The Tamil Nadu Dr.M.G.R Medical university, Chennai is the result of my original and independent research work carried out under the guidance of **Dr. C. SENTHIL KUMAR M.Pharm.,Ph.D.** Associate Professor, Department of Pharmacology, Karpagam College of Pharmacy, Coimbatore-32, & Co-Guide Dr. P. KRANTIKUMAR, **SURAS LABORATORIES,** during the academic year 2016- 2017.

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Examination centre:

Date:

Internal Examiner

Convener of Examination

External examiner

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Mr. MUHAMMED THWAYYIB.PK

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LIST OF ABBREVATIONS

S.NO	SHORT FORM	ABBREVATIONS
1	PBS	Phosphate Buffere Solution
2	DPPH	2,2-Diphenyl-1-picrylhydrazyl
3	BHA	Butylated Hydroxyanisole
4	BHT	Butylated Hydroxytoluene
5	HDL	High Density Lipoprotein
6	LDL	Low Density Lipoprotein
7	VLDL	Very Low Density Lipoprotein
8	TC	Total Cholesterol
9	TG	Triglyceride
10	WHO	World Health Organisation
11	ATP	Adenosine Triphosphate
12	IC-50	Inhibitory Concentration
13	OECD	Organisation For economic cooperation and development
14	OGTT	Oral Glucose Tolerance Test
15	PP	Post Parturition
16	NO	Nitric oxide
17	EC	Echitamine Chloride
18	CCL_4	Carbon Tetra Chloride
19	CMC	Carboxy Methyl Cellulose

20	PGE2	Prostaglandin E_2
21	Bap	Benzo(a)pyrene
22	H_2SO_4	Sulfuric acid
23	HCO_3	Bicarbonate
24	NMR	Neuclear Magnetic Resonance
25	NAOH	Sodium Hydroxide
26	DM H_2O	Demineralization Water
27	OGTT	Oral Glucose Tolerance Test
28	IAEC	Institutional Animal Ethics Committee
29	NEDA	N-(1-Naphthyl)Ethylenediamine Dihydrochloride
30	COX-1	Cyclooxygenase-1
31	COX-2	Cyclooxygenase-2
32	MDA	Malondialdehyde
33	CPA	Co-polymer alloy
34	MDA	MethyleneDianiline
35	SOD	Superoxide Dismutase
36	HMG-COA	3-Hydroxy-3-MethylGlutaryl-Coenzyme A
37	MN	Manganese
38	DNA	DeoxyriboNucleic Acid
39	GAE	Gallic acid equivalents
40	ASE	Accelerated Solvent Extraction
41	EtOAc	Ethyl acetate

42	LC-50	Lethal concentration,50%
43	EAC	Extruded activated carbon

1. INTRODUCTION

Hyperlipidemia is a disorder of lipid metabolism manifested by increase of plasma concentrations of the various lipid and lipoprotein fractions such as increase of serum total cholesterol (TC), low-density lipoprotein (LDL), triglyceride (TG) concentrations, and a decrease in the high-density lipoprotein (HDL) concentration. Hyperlipidemia is the key risk factor for cardiovascular disorders and has been reported as the most common cause of death in developed as well as developing nations. Hyperlipidemia may be caused by specific genetic abnormalities called primary hyperlipidemia6 or may be idiopathic caused by lifestyle habits or medical diseases such as diabetes, kidney disease, pregnancy, hypothyroidism and heart disease.

Hyperlipidemia prevalence continued to increase annually, requiring the develop-ment of drugs capable of lowering blood lipids to reduce mortality and morbidity due to cardiovascular complications. Although synthetic lipid-lowering drugs are useful in treating hyperlipidemia, there are number of adverse effects. So, the cur-rent interest has stimulated the search for new lipid-lowering agents with minimal side effects from natural sources.

Herbal medicines are the oldest remedies known to mankind. Herbs had been used by all cultures throughout history. In the last few years, there has been an exponential growth in the field of herbal medicine and these drugs are gaining popularity both in developing and developed countries because of their natural origin and less side effects when comparing other system of medicine. India being the botanical garden of the world with more than 2400 medicinal plants out of 21000 species being listed by WHO, is the largest producer of medicinal plants around the globe.

Alstonia scholaris is a large, evergreen tree, 10-15m in height, indigenous to the evergreen forests at altitude of 450-1,200m and cultivated throughout the hotter parts of India. Stem of this plant is straight rough whereas bark is green or black, 1.25cm thick, exuding milky latex, leaves broad obovate, elliptic, decurrent, glabrous, entire inflorescence solitary axillaries, cauliforous and ramflours on short leafy shoots. Male head is sessile or on short peduncles receptacles, sometimes born on the ultimate twing, Female head are oblong ovoid receptacle, syncarpus, cylindrics. Seeds are separated horny endocarpus enclosed by sub-gelatinous exocarpus (1mm thick) oblong ellipsoid in

nature. The sweet yellow sheaths around the seeds are about 3-5 mm thick and have a taste similar to that of pineapple, but milder and less juicy. Even though it is well known for its antibacterial, anti-inflammatory, anti-diabetic, antioxidant and immunomodulatory properties there are no evidences regarding the anti-hyperlipidaemic effect of the stem hence our study has its relevance

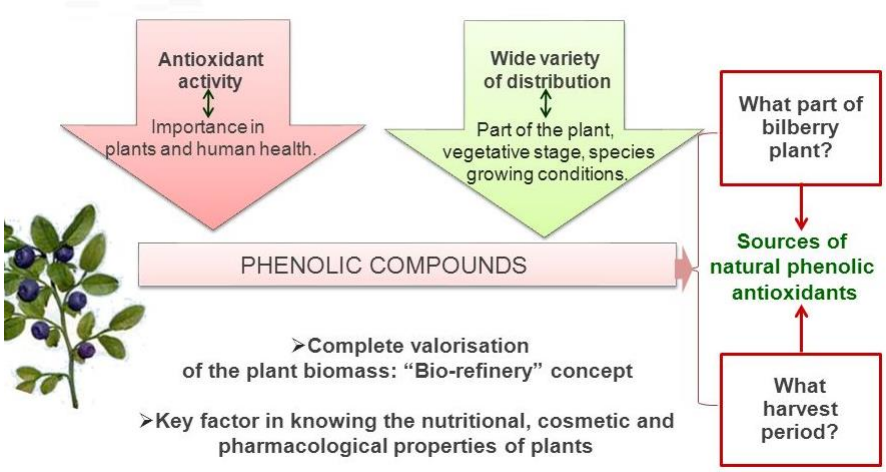

Fig 1. Antioxidant activity of flavonoids

The biggest organ in the body is the liver and it is likewise fills in as the essential metabolic organ of the body. In spite of the fact that the liver is comprised of various cells like hepatocytes, endothelial, kupffer and stellate cells are the most dominating with critical capacities. Another most essential one of a kind component of the liver is its capacity to recover. Well grown-up liver (i.e. Grown-up) is the standard organ accountable for detoxifying and metabolizing, exogeneous/endogenous mixes, rendering them more hydrophilic, which as often as possible impact their force and action1.

Liver infections are the genuine restorative issues went up against by the people wherever all through the world. The epidemiological review demonstrates that around 20,000 passings happen reliably in light of liver issue. In Africa and Asia, the major driver of liver maladies are contaminations by infection and parasite, while in Europe and in North America, a vital reason is liquor manhandle. Liver ailments are primarily realized by deadly chemicals, over the top affirmation of ceaseless liquor, diseases and immune system issue. Hepatic harm by over measurements of drug appears, from every angle, to be a run of the mill contributing component. Liver is required to do physiological limits and additionally to guarantee against the perilous of dangerous drugs and chemicals. Prescription impelled substance damage is accountable for 5% of each

mending focus attestation and half of all serious liver disappointment. Over 75% of episodes of specific prescription reactions achieve liver transplantation or death2.

Pathophysiological Mechanisms

Pathophysiological components of hepatotoxicity are as yet being found and contain both hepatocellular/extracellular systems.

Disturbance of hepatocyte: Medications can bound to intracellular proteins by covalent tying which realize a reducing in ATP levels inciting actin intrusion. Some portion of actin fibrils at the surface of the hepatocyte causes blebs and burst of the layer.

Plants plays a vital role in maintaining human health and improving the quality of human life from thousands of years and serves to human the valuable components of medicines, seasonings, beverages, cosmetics and dyes. Herbal medicine contains natural substances that can promote health and reduce illness. Now days researchers gives main focus on plant research has increased all over the world and a large body of evidence has been collected to show immense potential of medicinal plants used in various traditional systems. Furthermore many western drugs had their origin in plant extract. There are many herbs, which are used to treat cardiovascular problems, liver disorders, central nervous system, digestive and metabolic disorders. They give their potential to produce significant therapeutic effect and can be used as drug or supplement in the treatment, management of various diseases. Herbal drugs or medicinal plants, their extracts and their isolated compounds have exhibits spectrum of biological activities. The plant, *Alstonia scholaris,* invites attention of the researchers worldwide for its pharmacological activities ranging from antimalarial to anticancer activities. *Alstonia scholaris* Linn. R.Br. belongs to family Apocynaceae, grows throughout India, in deciduous and evergreen forests and also in plains. The plant is found in India in the sub Himalayan region from the Yamuna eastward ascending to 3000 feet above sea level, abundantly found in West Bengal and South India. It has wide occurrence also in the Asia-Pacific region from India, Sri Lanka through mainland South-East Asia and Southern China, throughout Malaysia to northern Australia and Solomon Islands. The timber is a non-durable hardwood, suitable for light indoor construction purposes, pulp and paper production. The wood has been used for school blackboards, hence the name ‘scholaris’. The bark is official in the Indian, British and French Pharmacopoeias. The plant is a large evergreen tree up to 17 to 20 m in height with a straight often fluted and buttressed bole, about 110 cm in diameter. Bark is grayish

brown, rough, lenticellate abounding in bitter, white milky latex; leaves 4-7 in a whorl, coriaceous, elliptic-oblong, pale beneath; flowers small, greenish white, numerous in umbellate panicles, corolla tube short, very strongly scented; fruits follicles, 30 – 60 cm long; seeds papillose with brownish hair at each end . The synonyms of the Alstonia Scholaris include Echites scholaris L. Echites pala Ham., Tabernaemontana alternifolia Burm. The plant is also known as Alipauen, Andarayan, Bita, Dalipauen, Dirita, Dita, Ditaa, Dilupaon, Lava, Lipauen, Oplai, Pasuit, Pulai, Tanitan, Tangitang, Milky pine, White chesse wood, Devil tree, Shaitan wood, Saittan ka jat, Hale, Satween, Elilappalai, Saptaparna, Phalagaruda throughout the world. In the literature this plant is reported as a stimulant, carminative, stomachic, expectorant and febrifuge . The decoction of the dried bark is used extensively to treat asthma, hypertension, lung cancer and pneumonia, whereas an infusion of the leaves is used to cure fever, no systemic pharmacological studies regarding broncho-vasodilatory activity have been carried out. The present investigation was, therefore, undertaken to test this possibility on ethanol extract from leaves of Alstonia scholaris, and it comprised two parts. The first series of experiments was performed on anaesthetized rats (in vivo) to examine the effects of the extract on carbachol-induced respiratory and cardiovascular changes. The second series was performed in vitro using vascular, tracheal and gut tissues from rabbit and guinea-pig to assess the potential bronchodilatory and other antagonistic effects of the extract on carbacholinduced hypotension and bradycardia. In Ayurveda, it is reported that the bark of the plant when soaked in water overnight, can reduce the 9 blood glucose level after oral administration however no much characterization of this activity has been done on scientific basis. We therefore subjected the aqueous extract of bark of Alstonia scholaris L. to preliminary photochemical investigation which showed presence of alkaloids, tannins, flavonoids, saponins, glycosides and triterpenoids. The photochemical are indicative of its potential in the treatment of diabetes mellitus hence we undertook the present work to study the chronic antidiabetic effect and antihyperlipidemic effect of the bark extract in healthy and streptozotocin diabetic rats with the objective to focus on mechanism underlying the activity.

Profile of *Alstonia scholaris*

Local Names Bengali (satiani,chattin,chatium); Burmese (lettok); English (white cheesewood,birrba,milkwood pine,milk wood,milky pine,black board tree,devil's tree,dita bark); Filipino (dita,dalipoen); Gujarati (satuparni); Hindi (chatian,satni,satwin,saitan-

kijhad); Indonesian (rite,pulai,pule); Javanese (pule); Lao (Sino-Tibetan) (tinpet); Malay (pulai,pulai linlin); Nepali (chhatiwan,chhataun); Sanskrit (saptaparna); Tamil (elalaipalai,palegaruda,pala); Thai (sattaban,teenpet,teenpethasaban); Trade name (pulai,shaitan wood,chatiyan wood,white cheese wood); Urdu (chatiana); Vietnamese (caay suwxa,caay mof cua).

Botanic Description

Alstonia scholaris is a medium to large tree, to about 40 m high with a somewhat tessellated corky grey to grey-white bark. The boles of larger trees are strongly fluted to 10 m. The outer blaze is cream to yellowish in colour with abundant, milky latex that flows rapidly when cut. Leaves in whorls of 4-8 in the upper axils; leaf stalks 1-1.5 cm long, the lamina obviate to elliptical or elliptical-lanceolate, glabrous or sparsely hairy, tapering towards the base, 11.5-23 x 4-7.5 cm. Upper surface is dark green, the lower green-white with 25-40 pairs of lateral veins on each side of the midrib and 2-6 mm apart. The tip of the leaf is rounded or shortly pointed, tapering towards the base. The inflorescence is a much-branched terminal panicle, up to 120 cm long; flowers 7-10 mm long white, cream or green; the tube hairy; lobes sparsely or densely pubescent, 1.5-4 mm long, the left margins overlapping; strongly perfumed. Fruit a pendulous, two-lobed, dehiscent follicle, brown or green, dry or woody, spindle-shaped, 15-32 cm long, 4-6 mm in diameter, containing numerous flat, oblong, brown seeds, 4-5 x 0.9-1.2 mm, with a tuft of hairs 7-13 mm long at each end. The seed does not taper to a point at either end. Alstonia is named after Dr C. Alston (1685-1760), a professor of botany at Edinburgh University. The specific name scholaris is derived from the use of the wood for school boards in Myanmar. Biology The trees are often deciduous at irregular intervals. They do not flower at every leaf-change, but only after marked periods of dry weather. The large branches provide favourable nesting sites for wild bees. Pollination is by insects; when flowering, butterflies and bees often surround trees. The fruits open on the tree and the seeds, which have a tuft of silky hairs at each end, are dispersed by wind. Ecology In its natural range in Australia, it is a dominant canopy species found in coastal mesophyll vine forest with a canopy height of 35-42 m, in palm- dominated forests and in notophyll vine forests, associated with Argyrodendron peralatum, Castanospermum australe and Cerapetalum sucirubrum. Biophysical Limits Altitude: 0-900 m, Mean annual temperature: 12-32 degree Celsius, Mean annual rainfall: 1200-1400 mm Soil type: Favorable soils include alluvia, basaltic red earth, yellow earth with grey- brown topsoil,

stony red earth on basic volcanic soils, sandy grey earth, brown earth from a volcanic mixture of rocks and soils derived from metamorphic rocks.

Documented Species Distribution Native-Australia, Bangladesh, Brunei, Cambodia, China, India, Indonesia, Laos, Malaysia, Myanmar, Nepal, Papua New Guinea, Philippines, Solomon Islands, Sri Lanka, Thailand, Vietnam Exotic-Taiwan, Province of China, USA. Products Food: The latex provides a good quality chewing gum. Fuel*: A. scholaris* has been recommended as a fuelwood species for the patana lands of Sri Lanka. Fibre: Bark yields a fibre, and the wood is regarded as suitable for pulp and paper production. Timber: A. scholaris is the most important source of pulai timber. The density of the wood is 270-490 kg/cubic m at 15% mc. Heartwood cream to pale yellow, sapwood wide and visually indistinct from the heartwood. Often has strong odour and a bitter taste. It is used for pattern making, corestock, plywood, carving and mouldings. The wood is also used for making coffins in Sri Lanka and school boards in Myanmar. Essential oil: Flowers of *A. scholaris* yield an essential oil. Medicine: Australian aborigines used the bark for treatment of abdominal pains and fevers, the latex for neuralgia and toothache. In India, the bark is used to treat bowel complaints and has proved a valuable remedy for chronic diarrhea and the advanced stages of dysentery. Leaves used for treating beriberi, dropsy and congested liver. Other products: Wood charcoal is used as gun powder. Services Ornamental: The tree is sometimes planted as an ornamental. Other services: In a study of the ethno botany of the Nagas of Nagaland in northeast India, *A. scholaris* was amongst the native plants used in magicoreligious beliefs. Tree Management Regular dry season watering is essential for good growth, and deep mulch has proved beneficial to young trees. It has been managed as a fuel wood species in Sri Lanka under a short coppice rotation of 6-8 years. In a social forestry planting in India, the species reached 3.6 m height and 10 cm diameter at 3.5 years in mixed species. In plantations in Taiwan, it reached an average of 23.5 m in height and 51 cm dbh in 18 years. A maximum of 35 m in height and 109 cm dbh was attained at 41 years of age. Germplasm Management Seeds can be stored in closed tins for 2 months, maintaining a germination rate of 90%. Based on the seed size, this species may show orthodox seed storage behaviour. There are approximately 357 000 seeds/kg. Pests And Diseases A leaf skeletonizer, Parotis marginata, causes significant damage to nursery stock and young plantations. The timber is liable to termites, pinhole and marine borers, while the sapwood is highly susceptible to lyctid borers.

Phytochemistry

Alstonia scholaris Linn. is known to be a rich source of alkaloids and there is interest among the scientist to use this for therapeutic purposes. Amongst the chemical classes present in medicinal plant species, alkaloids stand as a class of major importance in the development of newer drugs because alkaloids possess a great variety of chemical structures and have been identified as responsible for pharmacological properties of medicinal plants. However, of the large variety of the alkaloids (about 180 alkaloids) isolated, so far only few have been assessed for biological activities. Almost all the parts of plant (bark, flower, root) are found to contain active principles. The species A.scholaris is used in commercial formulation Ayush. The bark of this plant contains alkaloid ditamine and echitamine, echitenine, echicaoutchin, an amorphous yellow mass, echicerin in acicular crystals, echitin in crystallized scales, echitein in rhombic prisms (a crystallisable acid) and echiretin an amorphous substance, resembling an alkaloid, a fatty acid and fatty resinous substances. An uncrystallisable bitter principle called ditain was isolated and ascribed the febrifuge properties of the drug. Dung et al extracted the fresh plant material with hexane, hydro distilled the combined extracts in slight and wet residue and analyzed by a high-resolution GC and GC/MS. The principal constituents were reported to be linalool (35.7 %), cis and trans linalool oxides, alpha-terpineol and terpinen-4- ol. Atta-ur-Rahman et al reported the isolation of an anilinoacrylate alkaloid, scholaricine, from the leaves of *Alstonia scholaris* to which structure 2-(demethylschoarine) has been suggested. They also reported the isolation of 19, 20-dihydrocondylocarpine alkaloid from the leaves of *Alstonia scholaris* (8). Atta-ur-Rahman et al also isolated 19, 20-Z- Vallesamine and 19, 20-E- Vallesamine from *Alstonia scholaris* (9). Lagunamine (19-hydroxytubotaiwine), angustilobine B acid and losbanine (6,7-seco-6-norangustilobine B) were obtained from the leaves of Philippine A.scholaris, together with tubotaiwine, its oxide and 6,7- seco-angustilobine B. 17-OAcetylechitamine was isolated from the bark of the plant along with echitamine. Macabeo et al reported the isolation and structural elucidation (MS and NMR) of first seco-uleine alkaloids, manilamine (18-hydroxy-19,20-dehydro- 7,21-seco-uleine) *Alstonia scholaris* and N4-methyl angustilobine B) from the (pH 5) alkaloid extract of Philippine leaves together with the known indole alkaloids 19,20-(E) vallesamine, angustilobine B N4- oxide, 20(S)-tubotaiwine and 6,7-seco-angustilobine B. Tatsuo Yamauchi et al isolated several alkaloids from the leaves of *A. scholaris*. 19-

epischolaricine, Nb-methyl scholaricine, Na-methylburnamine and vallesamine Nb oxide were isolated and their structures were determined by spectral and chemical methods. They reported that the leaves of plants from Taiwan and Thailand showed similar alkaloid patterns, with picrinine, nareline and alschomine as the major alkaloids. Indole alkaloids, nareline ethyl ether, 5-epi-nareline ethyl ether and scholarine-N4-ioxide, in addition to nareline methyl ether, picrinine and scholaricine were isolated from the leaf extract of A. scholaris. Another indole alkaloid, alstonamine and a sitsrikine type indole alkaloid, rhazimanine, were also isolated from the leaves of *Alstonia scholaris.*

Pharmacology

Traditional the bark is bitter, astringent, acrid, thermogenic, digestive, laxative, anthelmintic, febrifuge, antipyretic, depurative, galactogogue, stomachic, cardiotonic and tonic. It is useful in fever, malarial fever, abdominal disorders, diarrhoea, dysentery, dyspepsia, leprosy, skin diseases, pruritus, tumours, chronic and foul ulcers, asthma, bronchitis, cardiopathy, helminthiasis, agalactia and debility. The milky exudate is bitter and is good for ulcers, vitiated conditions of vata and otalgia. The preparation infusion, 1 to 2 ozs., of tincture, 1 to 2 drachms diluted in water and of ditanin 5 to 10 grains given two or three times a day and an extract is prepared from the fresh bark and given in milk in cases of leprosy. It is also used as an anthelmintic. Milky juice is applied to ulcers and to rheumatic pains; mixed with oil and dropped into ear it relieves earache. Tincture of the bark acts in certain cases as a powerful galactogogue. Juice of the leaves with that of fresh ginger-root or zedoary is administered to women after confinement. The drug is also used in cases of snake-bite. The active constituents of the plant include antimalarials, CNS depressants, anticancers, antituberculosis, antidysentrics and galactopoietics.

Scientifically Validated Uses

Antimicrobial activity property of the plant constituents of *A. scholaris* (alkanes, alkanols and sterols). Evaluated the antibacterial activity of the petrol, dichloromethane, ethyl acetate, butanol fractions of crude methanolic extracts of the leaves, stem and root barks of *Alstonia scholaris* and reported that butanol fraction exhibited broader spectrum of antibacterial activity. Antidiarrhoeal activity The antidiarrhoeal effects of the aqueous and the alcoholic bark extracts of A. scholaris in mice were reported. Antiplasmodial activity evaluated the antiplasmodial activity of the methanolic extracts of various parts of *A. scholaris* which was tested against multidrugresistant K1 strain of Plasmodium

falciparum cultured in 73 human erythrocytes. Pronounced antiplasmodial activity was exhibited. The indole alkaloids were isolated from the active extract and were subsequently tested against the K1 strain of P. falciparum. They reported pronounced antiplasmodial activity mainly among the bisindole alkaloids, particularly villalstonine and macrocarpamine with IC50 values of 0.27 and 0.36 μM, respectively. Ironically Gandhi and Vinayak have reported that the petroleum ether extract and methanol extract of the bark of *Alstonia scholaris* were found to be devoid of antiamalarial activity in mice infected with Plasmodium berghei. However, they have noticed a dose-dependent improvement of conditions and delayed mortality amongst animals receiving methanol extract of *A. scholaris*. Reports state that *A. scholaris* has little or no demonstrable action in malaria induced in monkeys and naturally occurring in human patients. It cannot, therefore, be recommended as a substitute for quinine and other cinchona alkaloids. Hepatoprotective activity the hepatoprotective effect of *Alstonia scholaris* R. Br. On liver injuries induced by carbon tetrachloride (CCl_4), H- Dgalactosamine, acetaminophen and ethanol was investigated by Lin et al by serum-biochemical and histopathological examinations. All serological and histopathological effects of A. scholaris were comparative with those of Bupleurum chinense, which has been reported previously as treatment criteria of hepatitis. A tendency was also shown to inhibit cell necrosis and inflammatory cell infiltration caused by H-Dgalactosamine in histopathological examination. Anticancer activity Methanol extracts of root barks of Alstonia macrophylla, A. glaucescens, and A. scholaris, collected from Thailand, have been assessed for cytotoxic activity against two human lung cancer cell lines, MOR-P (adenocarcinoma) and COR-L23 (large cell carcinoma), using the SRB assay. Pleiocarpamine, O-methylmacralstonine and macralstonine were all considerably less active than villalstonine. Antimutagenic activity Lim et al reported the antimutagenic effect of *Alstonia scholaris* in micronucleus test in mice. Methylmethanesulfonate, mitomycin C and dimethylnitrosamine are genotoxic to bone marrow cells, since they fragment the chromatin material leading to the formation of micronucleated polychromatic erythrocytes in bone marrow cells of experimental mice. Expressions from *Alstonia scholaris* L. reduced the induction of micronucleated polychromatic erythrocytes by methylmethanesulfonate, mitomycin C and dimethylnitrosamine indicating that the plant has antimutagenic effect. The ASERS pretreatment increased the effect of radiation which was evidenced by enhanced cell killing when compared with the concurrent phosphate-buffered saline (PBS) treated irradiation group. Their study demonstrated that ASERS

treatment enhanced the effect of radiation and disease-free survival of the mice. They have also observed the alterations in the neoplastic activity of cyclophosphamide (CPA) by the extract of *Alstonia scholaris* (ASE) in mice transplanted with Ehrlich ascites carcinoma (EAC). Administration of *Alstonia scholaris* (120 mg/kg) 6 h before the administration of 25 mg/kg of CPA resulted in a greater tumor remission, drastic decline in the glutathione levels and increased the lipid peroxidation considerably when compared with drug alone. Jagetia et al studied the chemopreventive effect of various doses of hydroalcoholic extract of *Alstonia scholaris* (ASE) on the benzo(a)pyrene (BaP) induced fore stomach carcinoma in female mice. The pre or post-treatment of mice with 4 mg/ml ASE also significantly reduced the frequency of BaP-induced MN in the splenocytes of treated animals also reported the seasonal variation as well as cytotoxicity of different fractions of *Alstonia scholaris* . *Alstonia.* (ASE) against HeLa cells. The exposure of HeLa cells to different extracts prepared from the stem bark collected in monsoon, winter and summer seasons resulted in a dose dependent increase in the cell killing effect of ASE and they observed the highest cell killing effect for the extract prepared from the summer collections. Their study demonstrated that the extract prepared from the summer collection and the fractions containing the alkaloids were highly effective in cell killing. Teratogenicity: The teratogenic effect of hydroalcoholic extract of *Alstonia scholaris* (ASE) was studied in the pregnant Swiss albino mice on Day 11 of gestation. The litters were monitored regularly for mortality, growth retardation, congenital malformations, and appearance of physiological markers up to 7 weeks post-parturition (p.p.). The administration of 60, 120, 180, and 240 mg/kg ASE to the pregnant mice on day 11 did not induce mortality, congenital malformations, or alter the normal growth patterns. A further increase in the herbal extract dose up to 360 or 480 mg/kg resulted in a dose dependent increase in the mortality, growth retardation, and congenital malformations, characterized mainly by bent tails and syndactyly. The administration of higher doses (360 or 480 mg) of ASE also caused a significant delay in the morphological parameters such as fur development, eye opening, pinna detachment, and vaginal opening. The incisor eruption and testes descend were found to be delayed in litters born to the mothers treated with 240-480 mg/kg ASE. The study indicated clearly that ASE treatment caused teratogenic effect only at doses above 240 mg/kg. Lower doses had no developmental toxicity. Immunomodulatory activity The immunostimulating effect of *Alstonia scholaris* bark extracts was studied in BALB/c mouse. The aqueous extract at 100 mg/kg b.w. increased lytic activity of peritoneal exudate cells against Escherichia

coli. At the doses of 50 and 100 mg/kg b.w., the aqueous extract had no effect on primary antibody level. The aqueous extract at 50 mg/kg b.w. induced the cellular immune response while at 100 mg/kg b.w. inhibited the delayed type of hypersensitivity reaction. Antiasthmatic activity Bronchodilatory activity of the ethanol extract of *Alstonia scholaris* leaves in anaesthetized rats was reported. In vitro preparations of guinea pig trachea did not confirm this property, indicating that bronchodilation is not due to the direct tracheal smooth muscle relaxation. The vasodilatory activity of the extract was reported to be independent of adrenergic or muscarinic receptors or prostaglandins but was mainly via endothelial-derived relaxing factor, nitric oxide. The extract inhibited the spontaneous movements of rabbit jejunum and contractile effects of acetylcholine and histamine on guinea-pig ileum. Additionally, the extract caused marked reduction of barium chloride-, potassium chloride- and calcium chloride-induced contraction on guinea-pig ileum and pulmonary artery, implying a direct interference of plant extract with the influx of calcium ions into cells. However, the extract had no detectable effect on mobilization of intracellular calcium. These results coupled with the in vivo effects of ethanol extract reveal that the *Alstonia scholaris* leaves possess broncho-vasodilatory activity mediated presumably by prostaglandins, calcium antagonism and endothelium-derived relaxing factor(s). Anti-fertility activity the antifertility effect of *Alstonia scholaris* bark extract in male rats was evaluated by Gupta et al (30). Male Wistar rats were given with oral (200 mg/kg) bark extract of *Alstonia scholaris* 60 days. This did not cause body weight loss, while the weights of testes, epididymes, seminal vesicle and ventral prostate were significantly reduced. The production of step- 19 spermatids was reduced by 79.6% in treated rats. The population of preleptotene and pachytene spermatocytes was decreased by 61.9% and 60.1%, respectively. Spermatogonia and Sertoli cell population were also affected. There was a decrease in seminiferous tubule and Leydig cell nuclear area, sperm count, motility, protein and sialic acid content of the testes, epididymes, seminal vesicle and ventral prostate. *Alstonia scholaris* bark extract had a significant antifertility effect in male rats. Gupta et al reported the antifertility effect of lupeol acetate isolated from benzene extract of *Alstonia scholaris* in male albino rats, which further augmented their findings. Free Radical Scavenging Activity evaluated the plant extracts of 17 commonly used Indian medicinal plants for their possible regulatory effect on nitric oxide (NO) levels using sodium nitroprusside as a NO donor in vitro.

The potency of scavenging activity was reported to be as follows:

Alstonia scholaris > Cynodon dactylon > Morinda citrifolia > Tylophora indica > Tectona grandis > Aegle marmelos (leaf) > Momordica charantia > Phyllanthus niruri > Ocimum sanctum > Tinospora cordifolia (hexane extract) = Coleus ambonicus > Vitex negundo (alcoholic) > T. cordifolia (dichloromethane extract) > T. cordifolia (methanol extract) > Ipomoea digitata > V. negundo (aqueous) > Boerhaavia diffusa > Eugenia jambolana (seed) > T. cordifolia (aqueous extract) > V. negundo (dichloromethane/methanol extract) > Gingko biloba > Picrorrhiza kurroa > A. marmelos (fruit) > Santalum album > E. jambolana (leaf).

All the extracts evaluated exhibited a dose-dependent NO scavenging activity. The *A. scholaris* bark showed its greatest NO scavenging effect of 81.86% at 250 microg/mL, as compared with G. biloba, where 54.9% scavenging was observed at a similar concentration. Wound healing activity wound healing activity of the ethanol and aqueous extracts of *Alstonia scholaris* was tested against excision, incision and dead space wound models. The wound healing was assessed by the rate of wound contraction, period of epithelialisation, skin breaking strength, granulation strength, dry granulation tissue weight, hydroxyproline, collagen and histopathology of granulation tissue. Malondialdehyde level was also estimated to evaluate the extent of lipid peroxidation. The extracts promoted wound healing significantly in all the wound models studied. Increased rate of wound contraction, skin breaking strength, granulation strength, dry granulation tissue weight, hydroxyproline and collagen, decrease in the period for epithelialisation and increased collagenation in histopathological section were observed with extracts treated groups. The extracts also significantly decreased the levels of lipid peroxidation. Analgesic and anti-inflammatory activities. The effect of ethanolic extract of leaves of *Alstonia scholaris* was evaluated in experimental models of pain and inflammation. the leaf extract at 200 and 400 mg/kg showed significant decrease in acetic acid induced writhings in mice with a maximum of 65.76 % at 400 mg/kg. in hot plate method, the percentage of pain inhibition was found to be 73.90 % and 79.56 % with 200, 400 mg/kg of extract. There was a significant inhibition in carrageenan induced paw edema with 200 and 400 mg/kg of the extract. Anti-ulcer activity The ethanolic extract of leaves of *Alstonia scholaris* was evaluated for anti-ulcer activity by pyloric ligation method. The animals treated with the extract did not show ulcer, whereas the ulcer score was found to be significantly high inhibition of ATP production or accumulation of lactic

acid. The extract had significant anthelmintic activity and the possible mechanism of action may be by inhibition of energy metabolism (unpublished data of the author). Antioxidant activity The effect of ethanolic extract of *Alstonia scholaris* Linn. (Apocynaceae) on various in vitro antioxidant parameters was evaluated. Ethanolic extract of Alstonia scholaris had significant (DPPH.) free radical scavenging, metal ion chelating, hydrogen peroxide scavenging, superoxide anion radical scavenging and ferric thiocyanate reducing activities. Ethanolic extract of *Alstonia scholaris* Linn. was found to prevents lipid peroxidation and radicalic chain reactions. The results observed were comparable to that of BHA, BHT, lascorbic acid and tocopherol.

Pharmacological Activities of Isolated Constituents

Echitamine Chloride: Echitamine chloride (EC), an indole alkaloid, extracted from the bark of *Alstonia scholaris* has got highly promising anticancer effect. The effect of this drug on the microsomal drug detoxifying system was studied in sarcoma-180 induced mice. When given subcutaneously at a dosage of 5 mg/kg body weight, it was able to alter the impaired drug detoxifying system which was observed in the sarcoma-180 bearing mice . Further, echitamine chloride was also found to affect both cellular and mitochondrial respiration, leading to reduction of the cellular energy pool and thereby resulting in the loss of viability of S-180 cells. They have also reported the enhancement of the cytotoxic effects of echitamine chloride by vitamin A on in vitro Ehrlich ascites carcinoma cell culture. They report a tumoricidal action by a free radical dependent mechanism similar to that of adiramycin, mitomycin – C and bleomycin. The anticancer effects of echitamine chloride on methylcholanthrene-induced fibro sarcoma, which exhibited significant regression in tumor growth. The altered activities of plasma and liver transaminases and gamma-glutamyl transpeptidase and lipid peroxidation in fibro sarcoma have been corrected to near normal after echitamine chloride treatment. The decreased liver glutathione content and the lowered activities of glutathione peroxidase, superoxide dismutase and catalase have also been reversed to near normal after echitamine chloride treatment. Alstonine: The indole alkaloid alstonine has been identified as the major component of a plant-based remedy. In a preliminary evaluation done by Wright et al, alstonine demonstrated in vivo antimalarial activity. It is used in Nigeria to treat mental illnesses by traditional psychiatrists. Although it is certainly difficult to compare the very concept of mental disorders in different cultures, the traditional use of alstonine is remarkably compatible with its profile in experimental

animals. Even though alstonine in mice models shows a psychopharmacological profile closer to the newer atypical antipsychotic agents, it also shows important differences. Meldrum and Ozawa et al reported that alstonine possesses clear anxiolytic activity, mediated by 5-HT2A/2C serotonin receptors, suggesting effectiveness against negative symptoms of schizophrenia; It interferes with the glutamate system in a manner consistent with resulting beneficial effects for schizophrenia. According to the study of Costa-Campos et al, alstonine lacks the pro-convulsant property common to many antipsychotics, a considerable advantage for chronic use in general and epileptic schizophrenic patients in particular. The lack of direct effects on dopaminergic system suggests lack of significant extra pyramidal effects, the major drawback of many antipsychotic agents. Beljanski and Beljanski reported about the anticancer activity of alstonine which successfully treated a relatively important proportion of BALB/C mice inoculated with transplantable YC8 lymphoma ascites cells as well as Swiss mice bearing Ehrlich ascites carcinoma cells. Development of some solid tumours was only partially prevented by alstonine. Beljanski also reported the capacity of alstonine to distinguish cancer DNA from the healthy tissue DNA. It inhibits DNA in vitro synthesis when DNA from different cancerous tissues or cells is used as template. The reported inhibitory effect of alstonine is due to its capacity to form an alkaloid- cancer DNA complex.

When a compound of low solubility such as lipid is need to be extracted from a solid mixture a Soxhlet extraction can be carried out. The technique places a specialized piece of glassware in between a flask and a condenser. The refluxing solvent repeatedly washes the solid extracting the desired compound into the flask. The Soxhlet extraction method was described by Soxhlet in 1879, in this procedure; oil and fat from solid material are extracted by repeated washing/percolation with an organic solvent usually hexane or petroleum ether, under reflux in a special glassware. Soxhlet extraction is only required where the desired compound has a limited solubility in a solvent and the impurity is insoluble in that solvent.

Lipids are a group of substances that, in general, are soluble in ether, chloroform, and other organic solvents but are relatively insoluble in water. An accurate and precise quantitative analysis of lipids in foods is important not only for nutritional labeling, but also for determining whether the food meets the standards for identity and uniformity, and for understanding the effects of fats and oils on the functional and nutritional properties of foods. The validity of the fat analysis of a food depends on many factors, including

proper sampling and preservation of the sample before the analysis. Because of commercial regulations, it is important for food producers to be able to report fat content in a serving size of a food item.

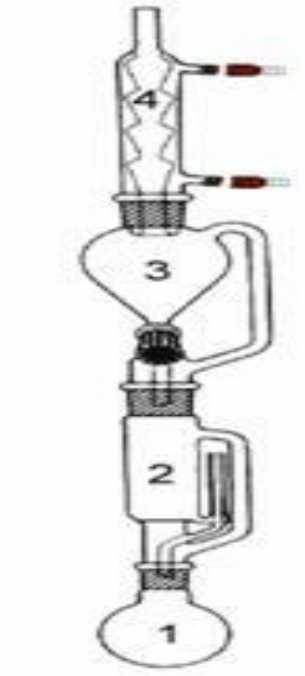

Fig 2. Soxhlet apparatus

1. Flask containing the solvent

2. Thimble placed in an extraction chamber

3. Funnel allows recovering the sample

4. Condensor

In this method the sample is dried, ground into small particles and placed in a porous cellulose thimble. The thimble is placed in an extraction chamber, which is suspended above a flask containing the solvent and below a condenser. The whole unit is supported with the heating unit. The flask is heated and the solvent evaporates and moves up into the condenser where it is converted into a liquid that trickles into the extraction chamber containing the sample. The extraction chamber is designed so that when the solvent surrounding the sample exceeds a certain level it overflows and trickles back down into the boiling flask. At the end of the extraction process, which lasts a few hours, the flask

containing the solvent and lipid is removed. In some device a funnel allows to recover the solvent at the end of the extraction after closing a stopcock between the funnel and the extraction chamber.

Safety cautions should be considered while using Soxhlet Extractor and Glass wares

To prevent the introduction of contamination into the sample and sample extracts at any time during the sample processing and analytical operation, it is vital that all glassware and other materials coming into contact with the sample should be clean properly.

All cleaned glassware should be stored prior to use under clean aluminum foil to prevent contamination by fallout from laboratory air, preferably in an enclosed cabinet.

To prevent the introduction of contamination to the cleaned glassware preparation and subsequent handling, it is important that suitable gloves should be worn. Disposable polyethene or latex gloves have been found suitable, however powdered gloves are not acceptable.

HYPERLIPIDEMIA

Hyperlipidemia is an umbrella term that refers to any of several acquired or genetic disorders that result in a high level of lipids (fats, cholesterol and triglycerides) circulating in the blood. These lipids can enter the walls of arteries and increase your risk of developing atherosclerosis (hardening of the arteries), which can lead to stroke, heart attack and the need to amputate. The risk of atherosclerosis is higher if you smoke, or if you have or develop diabetes, high blood pressure and kidney failure.

More than 3 million people have this genetic disorder in the United States and Europe. It is extremely common for those who live in developed countries and follow a Western high-fat diet.

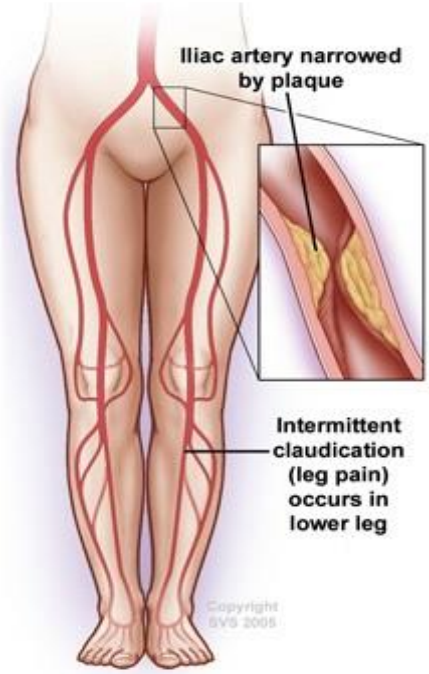

Fig 3. Susceptibility for atherosclerosis

Atherosclerosis is the abnormal accumulation of lipids and products resulting from an inflammatory response in the walls of arteries, and is the leading cause of death in the Western world. Heart attacks, angina pectoris, peripheral arterial disease, and strokes are common disease associate with atherosclerosis.

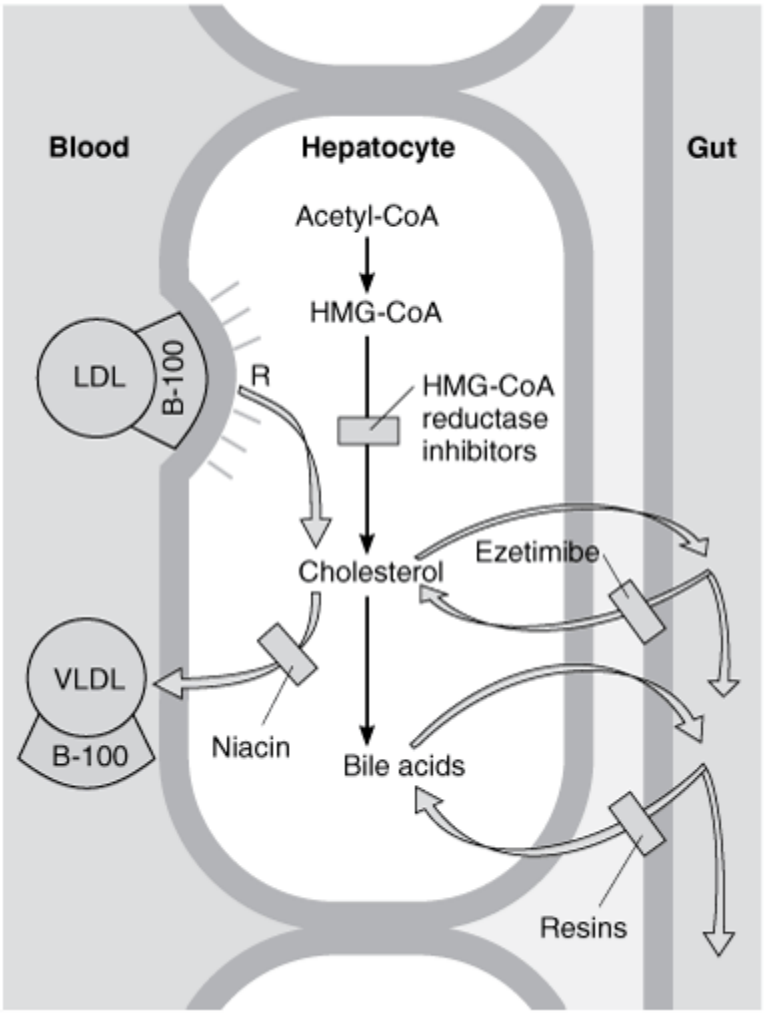

Fig 4. Formation of cholesterol

In some cases, lowering serum lipid concentrations has been shown to prevent the sequelae of atherosclerosis and decrease mortality in patients with a history of cardiovascular disease and hyperlipidemia. The five drug classes discussed in this chapter are used to decrease serum concentrations of lipids in the blood (hyperlipidemia) and to prevent or reverse associated atherosclerosis, or, in the case of hypertriglyceridemia, prevent pancreatitis. Although the drugs are generally safe and effective, adverse effects include drug–drug interactions and rare toxic reactions in skeletal muscle and the liver.

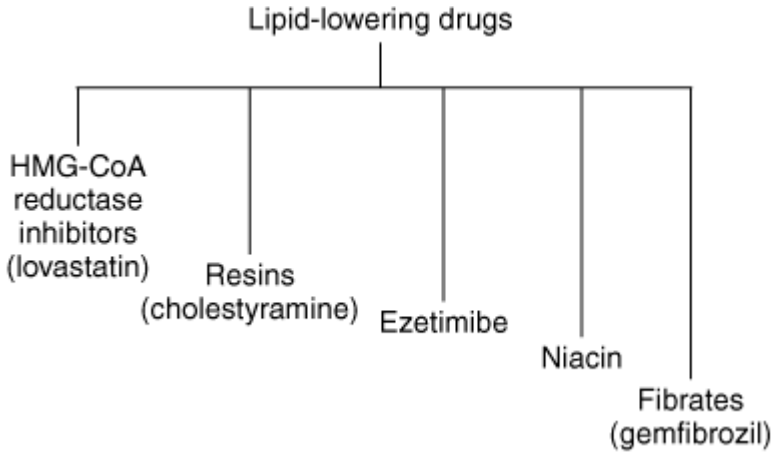

Fig 5. Classification of lipid lowering agents

Advantages of *Alstonia scholaris*

THERAPEUTIC POWERS OF ALSTONIA SCHOLARIS

This herb is best known for relieving ailments sucha as:

.Fever: The herb is a substituite for quinine and cinchinia.It is used widely for relieving the problems of intermittent and remittent fevers.

.**Bowel complaints:chhatim** is an effective compound that relieves bowel dysfunctions.

.diarrhoea and dysentery: The plant is effect in cases of chronic dysentery and diarrohea.

.Skin disorder:The extract from the tree of *Alstonia scholaris* is helpful in relieving acne,and ringworm.

.The *Alstonia scholaris* is used to improve appetite of new mothers.

.Chronic paludism and enlarged spleen can be relieved by using *Alstonia scholaris.*

.The fruits of the tree relieve insanity as well as epilepsy.

2. LITERATURE REVIEW

1.Mistry Dhruti et al ., 2**016**.Evaluate and compare qualitatively and quantitatively phytochemical constituents present in bark, stem and leaves of the medicinal plant *Alstonia scholaris*. Qualitative phytochemical screening revealed the presence of alkaloids, saponins, terpenoids, flavonoids, phenolic compounds, tannins, steroids, and glycosides in bark, stem and leaf extracts. The glycosides, alkaloids, gums and mucilage were found in higher quantity in bark of *A. scholaris* as compared to that in stem and leaf. Further, in vitro antioxidant potential of extracts from bark of *Alstonia scholaris* was also analyzed. Both aqueous and/or methanolic extracts from bark of A. scholaris showed potent total antioxidant activity. At every concentration studied, percentage of superoxide radicals scavenged by aqueous extracts from bark of A. scholaris was higher even than those of standard gallic acid at respective concentrations. Similarly the results of DPPH free radical scavenging assay showed that aqueous extracts from bark of A. scholaris better scavenged free radicals than the methanolic extract from bark of A. scholaris as well as standard Ascorbic acid tested at respective concentrations.

2.*Deepak Ganjewala* et al ., **2013**. Evaluated phytochemical composition, antibacterial and antioxidant properties of methanolic extracts of different parts viz., leaves, follicles and latex of Indian devil tree (*Alstonia scholaris* Linn.) R. Br.Antibacterial activities of the methanol extracts against Gram +ve (Bacillus subtilis and Staphylococcus aureus) and Gram -ve (Escherichia coli, Pseudomonas aeruginosa) bacteria were determined by well diffusion techniques. Aantioxidant profiles of methanol extracts were determined by 1,1-diphenyl-2-picryl-hydrazil (DPPH) free radical scavenging, superoxide anion radial scavenging and ferric thiocyanate reducing assays. Phytochemical composition revealed abundance of flavonoids (97.3 mg QE/g DW), proanthocynidins (99.3 mg CE/g DW) and phenolics (49.7 mgGAE/g DW) in the leaf extract. Extracts of follicles and latex had comparatively very content of phenolics, flavonoids and proanthocyanidins. However, in follicle extract level of proanthocyanidins was significantly higher (46.8 mg CE/gDW). Latex extract among others exhibited most potent antibacterial activity. All the extracts displayed strong DPPH free radical and superoxide anion scavenging activities, only leaf extract displayed powerful reducing and ferrous ion chelating activities. Study revealed significant antioxidant activities of *A.*

scholaris leaf, follicles and latex extracts and potential antibacterial activity of latex extract.

3.*Ramachandra et al ., 2012*.Carried out to screen for total phenols, flavonoids, and free radical scavenging activity in methanolic extract of leaf, root and bark of *Alstonia scholaris* Linn (Apocynaceae) using in vitro tests including 1, 1-diphenyl-2-picryl-hydrazil (DPPH) free radical scavenging and superoxide anion radical scavenging methods. Significant differences in DPPH scavenging activity were found between methanolic extract of leaf, root and bark were investigated, the highest radical scavenging activity were observed in root extract (33.00±4.62% inhibition). The total phenol content of the investigated results ranged from 34.97± 0.76 to 46.11 ± 0.85mg GAE/g extract, while flavonoid content ranged from 14.43± 2.37 to 22.54 ± 0.98 mg QE/g extract and the antioxidant activity of the methanol extract increased in a concentration-dependent manner

4.*Bellah S F et al ., 2017*.Investigated for the assessment of the biological activities. The bark of *Alstonia Scholaris* were extracted with pet ether, chloroform, carbon tetrachloride and methanol extract to afford 0.9 g, 0.8 g, 0.7 g, 3 g respectively for the test. We used crude pet ether, chloroform and carbon tetrachloride extract of the plant for the screening of antimicrobial activity against some selected organisms as bacteria and fungi by disc diffusion method. Out of all samples, chloroform and carbon tetrachloride extract showed strongest zone of inhibition and spectrum of activity. In vitro antioxidant activity of the extract of *Alstonia scholaris* was estimated by using DPPH free radical scavenging assay method. In DPPH free radical scavenging assay IC50 value of methanolic extract of *Alstonia scholaris* was found to be 39 g/ml which indicates mild to moderate antioxidant activity while Ascorbic acid was the standard drug. In the bioassay of brine shrimp lethality, the methanol extract showed an average of LC50 0.91 µg/ml. This indicated that the cytotoxicity exhibited by methanolic extract was very significant.

5.*Phukan Parmita et al ., 2014*. Performed Phytochemical analysis of methanolic extracts of leaf, bark and latex of *Alstonia scholaris* (L) R.Br. revealed marked variation in overall content of phenolics in leaf, bark and latex extracts. The leaf extract had highest content of overall phenolics followed by bark and latex extracts. In the leaf extract, flavonoids and proanthocyanidins were present in abundance with values observed 89

mgQE/g DW and 92 mgCE/g DW, respectively, whereas the phenolics were only 49 mgGAE/gDW. In the bark extract, level of flavonoids and phenolics were comparatively lower than leaf extract, however proanthocynidins (66 mgCE/g DW) was found significantly higher. Latext extract had lowest content of phenolics (26 mgGAE/g DW), flavonoids (16 mgQE/g DW) and proanthocynidins (21mgCE/g DW). Methanol extracts of A. scholaris leaves, bark and latex extracts exhibited strong antioxidant activities in terms of scavenging DPPH free radicals. Antimicrobial microbial rtesponse have been observed.

6.Kausik *et al ., 2011*. Evaluated Complementary therapies based on herbal medicines are the world's oldest form of medicine and recent reports suggest that such therapies still enjoy vast popularity, especially in developing countries where most of the population does not have easy access to modern medicine. *Alstonia scholaris* (L.) R.Br (Apocynaceae) is an evergreen tropical tree native to Indian sub-continent and South East Asia, having grayish rough bark and milky sap rich in poisonous alkaloid. It is reported to contain various iridoids, alkaloids, coumarins, flavonoids, leucoanthocyanins, reducing sugars, simple phenolics, steroids, saponins and tannins. It has been reported to possess antimicrobial, antiamoebic, antidiarrheal, antiplasmodial, hepatoprotective, immunomodulatory, anticancer, antiasthmatic, free radical scavenging, antioxidant, analgesic, anti-inflammatory, antiulcer, antifertility and wound healing activities. In other parts of the world, it is used as a source cure against bacterial infection, malarial fever, toothache, rheumatism, snakebite, dysentery, bowl disorder, etc. Reports on the pharmacological activities of many isolated constituents from A. scholaris (L.) R.Br are lacking, which warrants further pharmacological studies.

7.*Kumar P et al ., 2011*. use of folkloric medicine has been the tradition of Indian therapeutics since time immemorial. Many plant and their parts have been used as a remedy for various diseases. Alstonia is one of the most important genus of Apocynaceae family to which many pharmacological activities can be attributed. The number of alkaloids obtained from plants like ditamine, echitamine have been used in various diseases like diarrhea, beri-beri, malaria and is till under detailed investigative study to bring about its potential medicinal properties. There are many reports about the various traditional uses to which this plant has been used for. Therefore this paper therefore aims to bring out the ethnobotanical uses of genus Alstonia with special significance to two most studies species viz. *Alstonia scholaris* and Alstonia boonei so as to provide better

scope of carrying out more in vivo experiments based on evidences presented in this review.

8.*Abhijit D et al ., 2011*. Reviewed that *Alstonia scholaris* is a traditionally important medicinal plant. This evergreen tree is native to the Indian subcontinent and Southeast Asian countries. The plant is used in traditional, Ayurvedic, Unani, Homoeopathy and Sidhha/Tamil types of alternative medicinal systems against different ailments such as asthma, malaria, fever, dysentery, diarrhea, epilepsy, skin diseases, snakebite etc. Among the phytochemicals, alkaloids are mostly reported. This review compiles reports on phytochemical and pharmacological aspects of *A. scholaris*.

9.*Jahans S et al ., 2009*. Studied that *Alstonia scholaris*, commonly known as sapthaparna, has been used for centuries in Ayurvedic medicine for treatment of various disorders. The objective of this study was to investigate the possible chemopreventive and anti-oxidative properties of this medicinal plant on two-stage process of skin carcinogenesis induced by a single application of 7, 12-dimethyabenz(a)anthrecene (100 lg/100 ll acetone), and two weeks later, promoted by repeated application of croton oil (1% in acetone/thrice a week) till the end of the experiment (16 weeks) in Swiss albino mice.The tumor incidence, tumor yield, tumor burden and cumulative number of papillomas were found to be higher in the carcinogen treated control (without ASE treatment) as compared to experimental animals (ASE treated). Furthermore, a significant increase in reduced glutathione, superoxide dismutase and catalase but decrease in lipid peroxidation was measured in ASE administered experimental groups than the carcinogen treated control. The present study demonstrates the chemopreventive potential of *Alstonia scholaris* bark extract in DMBA-induced skin tumorigenesis in Swiss albino mice.

10.*Surya H et al ., 2005.* Investigated medicinal plants from Lombok has resulted in the collection of 100 plant species predicted to have antimicrobial, including antimalarial, properties according to local medicinal uses. These plants represent 49 families and 80 genera; 23% of the plants tested positively for alkaloids. Among the plants testing positive, five have been selected for further investigation involving structure elucidation and antimicrobial testing on the extracted alkaloids. Initial work on structural elucidation of some of the alkaloids is reported briefly.

11.*Nilubon J et al ., 2007*. Explained α-Glucosidase inhibitors are used in the treatment of non-insulin-dependent diabetes mellitus. We attempt to isolate α-glucosidase inhibitors from 24 traditional Thai medicinal plant samples. Potent α-glucosidase inhibitory activity was found in aqueous methanol extract of dried Devil tree (*Alstonia scholaris*) leaves. Active principles against α-glucosidase, prepared from rat small intestine acetone powder, were isolated and identified. The structures of these isolated compounds were found to be quercetin 3-*O*-β-D-xylopyranosyl (1‴ → 2″)-β-D-galactopyranoside and (−)-lyoniresinol 3-*O*-β-D-glucopyranoside on the basis of chemical and spectral evidence. The latter exhibited an inhibitory activity against both sucrase and maltase with IC_{50} values of 1.95 and 1.43 mM, respectively, whereas the former inhibited only maltase with IC_{50}values of 1.96 mM. This preliminary observation will provide the basis for further examination of the suitability of *Alstonia scholaris*as a medicinal supplement that contributes toward the treatment and prevention of diabetes.

12.*Rahmatullah* et al ., *2012*.Examined endocrinological disorder arising from insulin deficiency or due to ineffectiveness of the insulin produced by the body. This results in high blood glucose and with time, to neurological, cardiovascular, retinal and renal complications. It is a debilitating disease and affects the population of every country of the world. Around 200 million people of the world suffer from this disease and this figure is projected to rise to 300 million in the coming years. The disease cannot be cured with allopathic medicine as the drugs used do not restore normal glucose homeostasis and moreover have side-effects. On the other hand, traditional medicinal practitioners of various countries claim to cure diabetes or at least alleviate the major symptoms and progression of this disease through administration of medicinal plants. The Garos are an indigenous community of Bangladesh, who still follow their traditional medicinal practices. Their traditional medicinal formulations contain a number of plants, which they claim to be active antidiabetic agents. Since observation of indigenous practices have led to discovery of many modern drugs, it was the objective of the present study to conduct a survey among the Marakh sect of the Garos residing in Mymensingh district of Bangladesh to find out the medicinal plants that they use for treatment of diabetes. It was found that the tribal practitioners of the Marakh sect of the Garos use twelve medicinal plants for treatment of diabetes. These plants were Lannea coromandelica, Alstonia scholaris, Catharanthus roseus, Enhydra fluctuans, Terminalia chebula, Coccinia grandis, Momordica charantia,

Cuscuta reflexa, Phyllanthus emblica, Syzygium aqueum, Drynaria quercifolia, and Clerodendrum viscosum. A review of the scientific literature demonstrated that almost all the plants used by the Garo tribal practitioners have reported antidiabetic and/or antioxidant properties and have enormous potential for possible development of new and efficacious antidiabetic drugs.

13.*Jian Hua et al ., 2010.*Illustrated *Alstonia scholaris* (Apocynaceae) has been historically used in "dai" ethnopharmacy to treat chronic respiratory diseases. The leaf extract, developed as a commercially available traditional Chinese medicine, used to release tracheitis and cold symptom, has also been prescribed in hospitals and sold over the counter in drug stores. The investigation evaluated the anti-inflammatory and analgesic activities of the ethanolic extract, fractions and main alkaloids of *Alstonia scholaris* leaf to provide experimental evidence for its traditional and modern clinical use. Besides, to discover the active fraction and components for further better use in Chinese medicine is hopeful. The leaf of *Alstonia scholaris* was extracted with ethanol and then separated into different fractions. Furthermore, alkaloids were isolated by phytochemical method. The analgesic activities were investigated using acetic acid-induced writhing, hot-plate and formalin tests in mice. The anti-inflammatory activities were carried out *in vivo* and *in vitro*, including xylene-induced ear edema and carrageenan-induced air pouch formation in mice, and COX-1, -2 and 5-LOX inhibition. It has been exhibited that the EtOAc and alkaloid fractions reduced acetic acid-induced writhing response in mice, significantly. The ethanolic extract, EtOAc and alkaloid fractions remarkably inhibited xylene-induced ear edema. Further investigation was focused on the alkaloids fraction and three main alkaloids isolated from the alkaloids fraction, in different animal models. Alkaloids reduced acetic acid-induced writhing response, and xylene-induced ear edema in mice. In the hot-plate test, alkaloids did not increase the latency period of mice obviously. In the formalin test, alkaloids did not inhibit the licking time in first phase, but significantly inhibited the licking time in second phase of mice. Alkaloids increased significantly SOD activity and decreased levels of NO, PGE2 and MDA significantly, in air pouch mice model. Moreover, some alkaloids isolated from the leaf of *Alstonia scholaris* exhibited inhibition of COX-1, COX-2 and 5-LOX *in vitro* anti-inflammatory assay, which supported alkaloids as the bioactive fraction.

14.*Jian Hua et al ., 2010.*Illustrated *Alstonia scholaris* (Apocynaceae) has been historically used in "dai" ethnopharmacy to treat chronic respiratory diseases. The leaf

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15.*Sinnathambi et al ., 2010.*Ritrated *Alstonia scholaris* Linn. (R.Br.,) has been used in traditional and folklore medicine for the treatment of diabetes. The aim of the present study was to evaluate the effect of ethanolic extract of the leaves of *A. scholaris* (known as EEAS) in streptozotocin-induced diabetic rats.The streptozotocin-induced diabetic rats were orally treated with vehicle (2% w/v Tween 80), glibenclamide (0.25 mg/kg) and EEAS (100, 200 and 400 mg/kg) to the respective treatment groups. The blood glucose level, body weight, glycosylated hemoglobin, muscle and liver glycogen, lipid profile, lipid peroxidation, antioxidant status were measured and histopathology of pancreas was performed after 6 weeks of treatment and compared to the control. EEAS and

glibenclamide were found to significantly (p<0.001) reduce the blood glucose level, glycosylated hemoglobin and lipid peroxidation, whereas they increased body weight, liver and muscle glycogen and antioxidant status. The antidiabetic effect was sustained from 1 week onwards till the end of the study. The histopathology of pancreas revealed that the pancreatic β-cell damage with streptozotocin did not reverse in any of the treatment groups.It has been concluded that EEAS, in addition to the antidiabetic activity, also possess antihyperlipidemic and antioxidant activities in the streptozotocin-induced diabetic model.

16.*Ganesh C et al ., 2004*.Explained the plant extracts of 17 commonly used Indian medicinal plants were examined for their possible regulatory effect on nitric oxide (NO) levels using sodium nitroprusside as an NO donor in vitro. Most of the plant extracts tested demonstrated direct scavenging of NO and exhibited significant activity. The potency of scavenging activity was in the following order: *Alstonia scholaris > Cynodon dactylon > Morinda citrifolia > Tylophora indica > Tectona grandis > Aegle marmelos (leaf) > Momordica charantia > Phyllanthus niruri > Ocimum sanctum > Tinospora cordifolia (hexane extract) = Coleus ambonicus > Vitex negundo (alcoholic) > T. cordifolia (dichloromethane extract) > T. cordifolia (methanol extract) > Ipomoea digitata > V. negundo (aqueous) > Boerhaavia diffusa > Eugenia jambolana (seed) > T. cordifolia (aqueous extract) > V. negundo (dichloromethane/methanol extract) > Gingko biloba > Picrorrhiza kurroa > A. marmelos (fruit) > Santalum album > E. jambolana (leaf).* All the extracts evaluated exhibited a dose-dependent NO scavenging activity. The *A. scholaris* bark showed its greatest NO scavenging effect of 81.86% at 250 mg/mL, as compared with G. biloba, where 54.9% scavenging was observed at a similar concentration. The present results suggest that these medicinal plants might be potent and novel therapeutic agents for scavenging of NO and the regulation of pathological conditions caused by excessive generation of NO and its oxidation product, peroxynitrite.

17.*Caixiang et al ., 2008*.Investigated the chemical constituents of Yunnan local medicinal plants *Alstonia scholaris*. Silica gel column chromatography was used to isolate the constituents, and spectroscopic techniques (NMR, IR, UV and MS) were used for structural elucidation. Four picrinine-type monoterpenoid indole alkaloids, 5-methoxyaspidophylline (**1**), picrinine (**2**), picralinal (**3**) and 5-methoxystrictamine (**4**) were obtained from the leaves of *Alstonia scholaris*. Compound **1** is a new monoterpenoid indole alkaloid.

18.*Dey et al ., 2011*.Clarified Alstonia scholaris is a traditionally important medicinal plant. This evergreen tree is native to the Indian subcontinent and Southeast Asian countries. The plant is used in traditional, Ayurvedic, Unani, Homoeopathy and Sidhha/Tamil types of alternative medicinal systems against different ailments such as asthma, malaria, fever, dysentery, diarrhea, epilepsy, skin diseases, snakebite etc. Among the phytochemicals, alkaloids are mostly reported. This review compiles reports on phytochemical and pharmacological aspects of A. scholaris.

19.*Kumar et al ., 2012*.Evaluate antidiabetic and hypolipidemic activities of *Kigelia pinnata* methanolic flowers extract in streptozotocin (STZ) induced diabetic wistar rat. Rats were made diabetic by a single dose of STZ at 60 mg/kg body weight *i.p.* The blood glucose level was checked before and 72 h after STZ injection to confirm the development of diabetes. The flower extract and glibenclamide were administered orally at the doses of 250 and 500 mg/kg body weight for 21 days. Daily oral treatment with the extract and standard drug for 21 days significantly reduced blood glucose, serum cholesterol and triglycerides levels. High density lipoprotein-cholesterol level was found to be improved (P<0.01) as compared to diabetic control group. It is concluded that *Kigellia pinnata* flowers extract have significant antidiabetic and hypolipidemic effect

20.*Mahendra et al ., 2014.*Alstonia scholaris* (L.) R. Br. and Alstonia macrophylla Wall. ex G. Don are two vital medicinal plant species (family: Apocynaceae). In India, the therapeutic use of Alstonia scholaris has been described in both codified and non-codified drug systems for the treatment of malaria, jaundice, gastrointestinal troubles, cancer and in many other ailments. Other species, Alstonia macrophylla has been used in conventional medicines in Thailand, Malaysia and Philippines as a general tonic, aphrodisiac, anticholeric, antidysentery, antipyretic, emmenagogue, and vulnerary agents. In India, Alstonia macrophylla is used as a substitute for Alstonia scholaris in various herbal pharmaceutical preparations. However, one certainly cannot evaluate the truthfulness of a practice (i.e. in scientific terms). In this article we discuss and summarize comparative data about traditional uses, phytochemistry, pharmacology and toxicity of *Alstonia scholaris* and Alstonia macrophylla. Moreover, in order to unfold future research opportunities, lacunae in the present knowledge are also highlighted. Literature about *Alstonia scholaris* and Alstonia macrophylla was collected by using electronic and library search. Additionally, referred

books on traditional medicine and ethnopharmacology were also utilized for receiving traditional records about both the plant species. Both *Alstonia scholaris* and Alstonia macrophylla are rich in different types of bioactive alkaloids. So far, broad spectrum of in vitro and in vivo biological and pharmacological activities have been reported to both the species. Amongst them, antimicrobial and anticancer activities were promising. The use of Alstonia macrophylla as a substitute for *Alstonia scholaris* is not at all justifiable as both the species are distinct from each other in their phytochemistry and pharmacology. Further detail chemical fingerprinting and metabolic studies of these two species are warranted to prevent their mutual adulteration most importantly in the context of commercial preparations.

21.*Bhanu et al ., 2013.*Herbal remedies have been employed in various medical systems for the treatment and management of different diseases. The plant *Alstonia scholaris* has been used in different system of traditional medication for the treatment of diseases and ailments of human beings. It is reported to contain various alkaloids, flavonoids and phenolic acids. It has been reported as bronchodilatory, antimicrobial, antiamoebic, antidiarrhoeal, antiplasmodial, hepatoprotective, immunomodulatory, anti-cancer, antiasthmatic, free radical scavenging, antioxidant, analgesic, anti-inflammatory, anti-ulcer, anti-fertility and wound healing activities. There are also reports available for the traditional use of this plant for its cardiotonic, anti-diabetic and anti-arthritic properties.

22.*Consolacion et al ., 2013*.Extracted of air-dry leaves of *Alstonia scholaris* (L.) R. Br. contains a mixture of cycloeucalenol (**1a**), cycloartanol (**1b**) and lupeol (**1c**); lupeol acetate (**2**); and betulin (**3**). The structures of these triterpenes were elucidated by extensive 1D and 2D NMR spectroscopy and confirmed by comparison of their ^{13}C NMR data to those reported in the literature. A previous study reported that the powdered leaves of *Alstonia scholaris* produced a highly significant decrease in blood glucose and a mechanism of this action was upon insulin triggering and direct insulin-like effects. Betulin and lupeol acetate were reported to exhibit hypoglycemic activity. Thus, only a mixture of **1a – 1c** was tested for hypoglycemic potential using the oral glucose tolerance test (OGTT). A possible hypoglycemic activity was observed for a mixture of **1a – 1c** at a dose of 25 mg/kg BW administered orally to normoglycemic mice.

23.*Deepti et al ., 2011*.Studied the antidiabetic and antihyperlipidemic effect of aqueous extract of *Alstonia scholaris* Linn bark in streptozotocin (STZ) induced diabetes in rats. The diabetes was induced by single dose of STZ (65 mg/kg) in citrate buffer, while the normal control group was given the vehicle (citrate buffer) only. After three days of induction of diabetes, the diabetic animals were treated further four weeks with aqueous extract of *Alstonia scholaris* bark (150 mg/kg and 300 mg/kg) and glibenclamide (4 mg/kg). Blood glucose estimation was performed every week of the study. At the end of study period, animals were sacrificed for biochemical studies. STZ-induced diabetic rats showed marked hyperglycemia, hypertriglyceridemia and hypercholesterolemia at the end of study period. Body weight and liver glycogen levels were reduced and glycosylated haemoglobin levels were significantly increased in diabetic rats. The four week treatment with aqueous extract of *Alstonia scholaris* bark (150 mg/kg and 300 mg/kg) significantly ameliorated the alterations in fasting blood glucose, serum triglyceride, serum cholesterol, liver glycogen. glycosylated haemoglobin and body weight in diabetic rats. Thus the present study suggested the potential of *Alstonia scholaris* bark in diabetes as well as related cardiovascular complications due to its antidiabetic and antihyperlipidemic properties.

24.*John Prosper et al ., 2012*.Explained *Alstonia scholaris* leaves and their aqueous and methanol extracts on blood glucose levels were studied in normal and alloxan-treated diabetic rabbits. Oral administration of the powdered leaves caused a time dependent glycaemia lowering effect in normal rabbits at all the dose levels studied (1.0, 1.5 and 2.0 g/kg) and in diabetic rabbits at the 2.0 g/kg dose only. The methanol extract also showed a glycaemia lowering effect in both normal and alloxan-diabetic rabbits while aqueous extract was effectives only in diabetic rabbits at a dose equivalent to 2.0 g/kg of the powdered leaves. The control drug, glimepiride (Amaryl®) was found effective in lowering the blood glucose levels in normal rabbits but was not effective in alloxan-treated diabetic rabbits. Leaves of the plant were found to contain elements like zinc, magnesium, manganese, iron, sodium and potassium. It is just possible that plant drug acts at least partially by providing some of these necessary elements to β-cells. The plant material may ultimately prove to be a relatively safe drug for human use, as it was found to be devoid of any adverse effects in rabbits and showed no mutagenic activity. *Alstonia boonei* De Wild is a herbal medicinal plant of West African origin, popularly known as God's tree or "*Onyame dua*". Within West Africa, it is considered as sacred in some forest

communities; consequently the plant parts are not eaten. The plant parts have been traditionally used for its antimalarial, aphrodisiac, antidiabetic, antimicrobial, and antipyretic activities, which have also been proved scientifically. The plant parts are rich in various bioactive compounds such as echitamidine, Nα-formylechitamidine, boonein, loganin, lupeol, ursolic acid, and β-amyrin among which the alkaloids and triterpenoids form a major portion. The present paper aims at investigating the main research undertaken on the plant in order to provide sufficient baseline information for future work and for commercial exploitation.

25.*Singh SK et al ., 2012.* Many aquatic snails act as intermediate hosts for the larvae of trematodes, Fasciola hepatica and Fasciola gigantica, which cause the diseases fascioliasis and schistosomiasis. The WHO has tested several thousands of synthetic compounds for the control of the snail host. Although effective, these molluscicides have so far not proved themselves to be entirely satisfactory. With a growing awareness of environmental pollution, efforts are being made to discover molluscicidal products of plant origin. Being products of biosynthesis, these are potentially biodegradable in nature. Several groups of compounds present in various plants have been found to be toxic to target organisms at acceptable doses ranging from <1 to 100 ppm. Common medicinal plants, i.e. Thevetia peruviana, *Alstonia scholaris* (Family; Apocynaceae), Euphorbia pulcherima and Euphorbia hirta (Family; Euphorbiaceae), have potent molluscicidal activity against freshwater snails. The toxicological actions of Thevetia peruviana may be due to the presence of apigenin-5-methyl ether (flavonoid) and triterpenoid glycosides, while a number of alkaloids (pseudo-akuammigine in addition to betulin, ursolic acid and beta-sitosterol), steroids and triterpenoids are present in Alstonia scholaris and the diterpenoids, pulcherrol, beta-sitosterol, hentriacontane, ellagic acid and beta-amyrin are present in Euphorbia hirta and in Euphorbia pulcherima. Although, at present very little literature is available on the control of vector snails through plant origin pesticides, an attempt has been made in this review to assemble all the known information on molluscicidal properties of common medicinal plants of eastern Uttar Pradesh, India, which might be useful for the control of harmful snails.

26.*Jai Bahadur Singh Kachhawa et al ., 2012*.Detailed *Alstonia scholaris* a potent therapeutic plant was used in the present study to evaluate the antibacterial activity against Grampositive bacteria i.e. Bacillus coagulans and gram negative bacteria i.e. Escherichia coli. Ciprofloxacin was used as standard drug, and methanol extract of

Alstonia scholaris bark at different concentrations were used for the experimental evaluations, where inhibition zones were calculated after disc diffusion assay for their antibacterial activity. Calculations of inhibition zones proved that *Alstonia scholaris* is a persuasive inhibitor against both the bacteria. Keywords: *Alstonia scholaris*, ciprofloxacin, methanol extract,

27.*Khan et al ., 2003*.Explained crude methanolic extracts of the leaves, stem and root barks of *Alstonia scholaris* and *Leea tetramera* on partitioning (petrol, dichloromethane, ethyl acetate, butanol) gave fractions exhibiting improved and broader spectrum of antibacterial activity. Especially the butanol fractions of *A. scholaris* and the root bark of *L. tetramera*. None of the fractions were active against the fungi tested.

28.*Antony et al ., 2013.*Investigated the antibacterial activity of leaves of *Alstonia scholaris* against bacterial pathogensby disc diffusion method, well method and incorporating the extract in the media before solidifying. The result of discdiffusiontechniqueshowed that fractions of leaf extract had pronounced antibacterial activity against MethicillinResistant Staphylococcus aureus (MRSA) and the clinical strain Providence stuartii. Antibacterial activity was alsotested againsta large group of Grampositive and Gram negative bacteria anditwas found to reside maximum in thebutanol and ethyl acetate fractions of methanol extract of leaf and bark.

PROFILE OF *ALSTONIA SCHOLARIS*

Taxonomical classification of A.scholaris Linn.R.Br

Taxonomy	:	*Alstonia scholaris*
Kingdom	:	Plantae, Planta
Subkingdom	:	Tracheobionta, vascular plants
Division	:	Magnoliophyta, Flowering plants
Class	:	Magnoliopsida, Dicotyledons
Subclass	:	Asteridae
Order	:	Gentianales

Genus Alstonia (devil tree) consists of about 40 -60 species, native to tropical and subtropical Africa, Central America, South East Asia, Polynesia and New South Wales, Queensland and North Australia, with most species in the Malaysian region. In India it is widely distributed in the Western Himalayas, Western Ghats and in the Southern region. Historically, the plant was scientifically named by Linnaeus as Echites scholaris. However to commemorate the great botanist Professor C.Alston, the generic name was changed to *Alstonia* by Robert Brown in 1811, where as the species name scholaris was retained to signify its use in schools in South East Asia, where the wood is traditionally.

Common names of *Alstonia scholaris*

Bengali : satiani, chatium, chattin

English : devil's tree, dita bark white cheese wood, birrba, milk wood pine, milky pine, black board tree

Gujarati : satuparni,

Hindi : chatian, satni, saitan-ki-jhad,

Sanskrit : sapthapama, saptapami and phalagaruda (sapta means 7 and pama or parni means leaves)

Tamil : elalaipalai, palegaruda, pala

Telugu : aedakularite chettu

Urdu : chatiana

Malayalam : Palamaram

Fig 6. Plant of Alstonia scholaris

Fig 7. Whole parts of *Alstonia scholaris* -I

Fig 8. Whole parts of *Alstonia scholaris* -II

Fig 9. Whole parts of *Alstonia scholaris* -III

Fig 10. Whole parts of *Alstonia scholaris* -IV

Fig 11. Whole parts of *Alstonia scholaris* -V

Fig 12. Whole parts of *Alstonia scholaris* -VI

Fig 13. Whole parts of *Alstonia scholaris* -VII

Fig 14. Whole parts of *Alstonia scholaris* -VIII

Identification and characterization of the plant

The identified tree *Alstonia scholaris* was medium sized, fifty to sixty feet high, evergreen tree (approximately fifty to fiftyfive years old), growing in the University College campus, Satavahana University, Telengana. The trunk was 50 to 70 cm in diameter, buttressed at base; bark thick, dark grey to grayish brown, somewhat rough, lenticellate, exuding plenty of milky latex.

Particulars	Parts
Simple, short petioled m whorls of four to seven, elliptic - oblong, lanceolate, four inches to eight inches long and one to two inches broad. Leaf margin was entire, coriaceous, upper surface bright green and shining, lower surface paler with whitish bloom, midrib prominent.	**Leaves**
Almost sessile or sub capitate, numerous in umbellate panicles, small about half an inch long, greenish white in colour, strongly scented, ypogynous, pentamerous, bisexual, regular, actinomot-phic arranged in corymbose umbellate cymes at the ends of branches.	**Flowers**
Two to three millimeters of an inch, gamosepalous, corolla amopetalous, salver shaped, eight to twelve millimeters long; throat of the corolla was villous or hairy.	**Calyx**
five in number, filaments very short, epipetalous, included within the corolla tube, disc annular or bibbed.	**Stamens**
Two, semi or sub apocarpous, ovaries distinct hirsute with a common style ending in an oblong or cylindric bifidstigma. Fruits had a pair of follicular mericarps, one to two feet long and three millimeters broad.	**Carpels**
Numerous, 30-60cm long, linear, flattened and slightly grooved with tufts of silky brownish hairs on both ends.	**Seeds**

Table 1. Plant morphology

3. AIM & OBJECTIVE

To evaluate the antihyperlipidemic and antioxidant activity of *Alstonia scholaris* stem extract on High fat diet-induced hypercholesterolemia and triton induced hyperlipidaemia models.To achieve this primary aim we fixed following objectives.

Objectives

1. To conduct a literature survey for establishing the relavence of the study.

2. To Collection and authenticate of *Alstonia scholaris* stem.

3. To successfully extract the dried stem of *Alstonia scholaris* using suitable solvents.

4. To evaluate toxicological profile of the extract.

5. To characterize the antioxidant property of the extract.

6. To Evaluate Antihyperlipidimic activity of Extract of *Alstonia scholaris* using high fat diet-Induced hypercholesterolemic and Triton Induced Hyperlipideamic models.

4. PLAN OF THE WORK

Plant Collection

↓

Plant Authenication

↓

Solvent Extraction

↓

Phytochemical Screening

↓

Acute Toxicological Studies

↓

Pharmacological Screening For Antioxidant Activity

- DPPH radical reducing activity
- Lipid peroxide assay
- Nitric Oxide scavenging assay
- Hydrogen peroxide assay

↓

Pharmacological Screening For AntiHyperlipidaemic activity

- High-cholesterol diet induced rat model
- Effect on Normocholesteremic rats

5. MATERIALS AND METHODS

SOLVENT EXTRACTION AND PHYTOCHEMICAL SCREENING

Plant collection and extraction *Alstonia scholaris* were collected from the regions of ananthagiri hills vikarabad, Telengana. After that the plant parts such as leaf and bark were coarsely powdered and subjected to successive solvent extraction using soxhlet apparatus.

Solvent extraction of samples

Alstonia scholaris leaf

Fresh mature leaves of *Alstonia scholaris* R.Br. tree were collected. They were cleaned, washed in water, dried at room temperature weighed and powdered. Weight of the dry powder was taken. The finely powdered leaves (100 gm) were taken in a 500 ml soxhlet apparatus and extracted with methanol for 24 hours. After extraction, the methanol extracts were dried free of solvent in a rotary evaporator at low temperature (40°C). The dried extracts were tested for anti-oxidant activities.

The methanol extracts of the plant powder leaf showed significant antioxidant activities and hence fractionation of the extracts was carried out. The methanol extracts of the leaves were suspended separately in water (lg extract in 50 ml water) and sequentially extracted with n-hexane (150 ml), chloroform (150 ml), ethyl acetate (150 ml) and butanol (150 ml). All the fractions except water fraction (obtained at the end of fractionation) were dried free of solvents in a rotary evaporator at low temperature (40°C) while aqueous fractions were freeze dried in a lyophilizer. The yield of each fraction was determined.

Testing for	Procedure	Results
Flavonoids	A few drops of NaOH ution was added to the extract ution (500f.l1) followed by dil. Cl.	The solution turned yellow d then colourless, indicating presence of flavonoids.
Alkaloids	Tested using 3 reagents mely Dragendorff reagent, Wagner's reagent and Mayer's reagent	A reddish brown ecipitate indicated the esence of alkaloids.
Phenols and Tannins	To the test solution (500f.l1), ew drops of FeCh were added.	Presence of phenols and nins was indicated by mation of a blue or blue-een colour solution.
Proteins	A few drops of 4% NaOH re added followed by 1 % $S0_4$	Violet or pink colour ution indicated the presence proteins.
Carbohydrates	To the extract solution 00µ1), 1 to 2 mL of Anthrone gent was added.	Formation of green colour ution indicated the presence carbohydrates.
Saponins	Few drops ofNa2HC03 were ded to the extract solution 00µ1) and shaken for 5 nutes.	Formation of froth or her indicated the presence saponins.
Glycosides	Few drops of aqueous NaOH re added to the extracts 00µ1).	Yellow colored solution dicated presence of ycosides.

Steroids	Chloroform was added to the tract solution (500µl) followed cone. H_2S0_4 added slowly ough the sides of the test tube.	The lower sulphuric acid ction turned brownish llow and the upper layer ned reddish orange which licated presence of steroids.

Table 2. Phytochemical Testing

ACUTE TOXICOLOGICAL STUDIES

ANIMALS

Healthy albino rats of either sex of 2-2½-months-old of body weight 125-150 g were housed in polypropylene cages at 25±2°C with light dark cycle of 12 h in the Animal House of the study center are to be used for the study. It should be acclimatized for seven days. All animals are to be given with standard rat feed and water ad libitum. The experiments were performed after approval of the protocol by the minute of Institutional Animal Ethics Committee (IAEC) and animal care was taken as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India.

ACUTE TOXICITY

The toxicity for the aqueous and ethanolic extracts stem of *Alstonia scholaris* was determined in albino mice, maintained under standard conditions. The animals were fasted overnight prior to the experiment. Fixed dose (OECD Guideline No. 423) method of CPSEA was adopted for toxicity studies.

STUDY DESIGN

Acute toxicity studies for the extracts were conducted as per OECD guidelines 423 using mice. Each animal was administered the extracts by oral route.

Acute Toxicity Studies

The aqueous extract of *Alstonia scholaris* L. bark was tested for its acute and short term toxicity in mice. To determine acute toxicity of the drug, overnight fasted wistar albino mice were orally fed with extract in increasing dose levels of 100, 500, 1000, 3000 and 5000 mg/kg body weight. The mortality and general behavior of the animals were

observed periodically for 48 h. The animals were observed continuously for the initial period of 4 h, intermittently for the next 6 h, then again at 24 h and 48 hrs following drug administration. The parameters observed were grooming, hyperactivity, sedation, loss of righting reflex and convulsions.

IN VITRO ANTIOXIDANT ACTIVITIES

DPPH RADICAL REDUCING ACTIVITY:

PRINCIPLE:

It is a rapid and simple method to measure antioxidant capacity. It involves the use of free radical, DPPH (2, 2- Diphenyl - 1- picrylhydrazyl) (Aquino et al, 2001). The odd electron in the DPPH free radical gives a strong absorption maximum at 517nm and is purple in color. The color turns from purple to yellow when the odd electron of DPPH radical becomes paired with hydrogen from a free radical scavenging antioxidant to form the reduced DPPH-H. The resulting decolourisation is stochiometric with respect to the number of electrons captured.

REAGENTS:

DPPH - 3mg in 25ml methanol (stored in dark bottle)

Methanol

PROCEDURE:

Freshly prepared DPPH (187 µl) was taken in different test tubes protected from sunlight. To this solution added different concentrations (0, 25, 50, 75,100,150,200µg/ml) of seed oil extract and fraction-IV. The volume was made up to 1ml with methanol. Keep the tubes in dark and after 20 min absorbance was measured at 515nm. Methanol was used as blank and vitamin C was used as positive control. The concentration of test materials to scavenge 50% DPPH radical (IC_{50} value) was calculated from the graph plotted with % inhibition against Concentration.

Lipid Peroxidation Activity

The stocking solutions, various working conc. were produced to get concentration of 25, 50, 75, 100, 150 & 200 µg/ml with distilled water. The standard stock solution was prepared by dissolving ascorbic acid (Standard Sample) in suitable solvent (methanol) with a final concentration of 1000 µg/ml and different concentration of 25, 50, 75, 100, 150 & 200 µg/ml were prepared by distilled water.

Potassium hydrogen phosphate (0.19 gram) was mixed with 8 gm of sodium chloride. To this 2.38 gm of disodium hydrogen phosphate was dissolved and made up to 1000 milliliter alongside DM H_2O and pH was adjusted to 7.4. To a set of eight clean dry test tubes, 2 ml of 0.25Mm HCL containing 15% trichloroacetic acid and 0.38% thiobarbituric acid were added and to this 1 ml of different concentrations of the test extracts were added. For five minutes the sample was kept. Centrifugation was done and absorbance of the upper layer was measured at 538 nm and the lipid peroxide content was found. All experiments were performed in triplicate.

Nitric Oxide Scavenging Method

The stocking solutions, various working conc. were produced to avail concentration of 25, 50, 75, 100, 150 & 200 µg/ml with distilled water. The standard stock solution was prepared by dissolving ascorbic acid (Standard Sample) in suitable solvent (methanol) with a final concentration of 1000 µg/ml and different concentration of 25, 50, 75, 100, 150 & 200 µg/ml were prepared by distilled water. Sodium nitroprusside 5mM in phosphate buffer at pH 7.4 saline was added with a range of concentrations of the test sample or standard and incubated at 25ºC for 150 minutes. At regular intervals, 1.5 ml of samples (incubated test sample) were taken off and a poured with 1.5 ml Griess reagent (1% Sulphanilamide, phosphoric acid (2 percent), and 0.1 percent NEDA 2.HCL.

The absorbance was read at 540 nm. The difference in the absorbance between test and control on nitric oxide was determined and depicted as percent scavenging of NO radical. Capability to scavenge the NO radical was designed by using standard formula .All experiments were performed in triplicate.

Hydrogen Peroxide Method

The stocking solutions, various working conc. were produced to get concentration of 25, 50, 75, 100, 150 & 200 µg/ml with distilled water. The standard stock solution was prepared by dissolving ascorbic acid (Standard Sample) in suitable solvent (methanol) with a final concentration of 1000 µg/ml and different concentration of 25, 50, 75, 100, 150 & 200 µg/ml were prepared by distilled water. 1 ml of standard and test solution was added to 0.6 ml hydrogen peroxide solution. After 10 minutes the reading of the solution was read at 230 nanometer using UV/VIS spectrophotometer alongside a blank containing PBS without H_2O_2.

The percentage scavenging of hydrogen peroxide of both plant fraction and standard compound were determined. The percentage inhibition was calculated to tests & standard making usage of the following formula. All experiments were performed in triplicate.

Calculation:

$$\% \text{ inhibition} = \frac{OD \text{ of control} - OD \text{ of sample}}{OD \text{ of control}} \times 100$$

Anti hyperlipidaemia Activity

High – Cholesterol Diet Model

Swiss albino rats weighing (200 – 250gm) were used for a standard experimental method as high cholesterol diet consisting of Cholesterol (1%), sodium cholate (0.5%), sucrose (30%), casein(10%), butter (5%) and standard chow diet (53.5%) for 7 days. The animals divided into four groups of control, test(150mg,300mg) and standard drug treated animals. The studies conducted in two stages. In the preliminary stage effective hypolipedemic doses of test(150mg,300mg) and standard drugs are worked out and in the final stage the effect of test and standard drugs are studied. The lipid profile includes total cholesterol LDL, HDL, VLDL and triglycerides were studied. The blood samples were collected after 6, 24 and 48 hour of drug administration.

Group 1: Control

Group 2: 150mg extract

Group 3: 300mg extract

Group 4: Standard Fenofibrate

Triton Induced Hyperlipidemic Rats

PROCEDURE

Eight week old adult male albino rats of Wistar strain, weighing approximately 150 to 200 g, were acclimatized for 7 days at room temperature (22±2ºC) and humidity of 45-64% in a 12- hour light/dark cycle in a room under hygienic condition. They were given access to water and a commercial diet ad libitum. The experiments were carried out in the Suran labs, Hyderabad, as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India, and approved by the Institutional Animal Ethics Committee (IAEC).

Chemicals

Triton WR-1339 (A non-ionic detergent, Isooctyl poly oxyethylene phenol) was obtained from Sigma Chemicals Co, Mumbai.

Induction of hyperlipidaemia

Hyperlipidaemia was induced in Wistar rats by intraperitoneal (i.p) injections of Triton WR-1339 at a dose of 400 mg/kg body weight. After 72 hours of triton injection received a daily dose of 5% CMC in 5ml/kg body weight for 7 days.

Experimental design

In the experiment, the rats were divided into three groups of eight rats each. Group I rats received 5% CMC and considered as controls, Group II rats were treated with Triton WR-1339 (400 mg/kg body weight with extract) and Group III rats were treated with Triton WR-1339 (400 mg/kg body weight with aqueous extract) and ethanolic extract of *Alstonia scholaris* stem (150mg & 300mg/kg body weight) and Standard fenobirate (100mg/kg body weight).

At the end of 8th day, rats were fasted overnight and sacrificed by cervical dislocation. Blood was collected, and serums were separated by centrifugation. Liver tissues were excised immediately and rinsed in ice-chilled normal saline, 500mg of the tissues were homogenized in 5.0 ml of 0.1 M Tris–HCl buffer (pH, 7.4). Biochemical

estimations were carried out in serum and liver tissues, parameters such as cholesterol, phospholipids, triglycerides , LDL, VLDL and HDL were analyzed.

Group 1: Control

Group 2: 150 mg extract

Group 3: 300 mg extract

Group 4: Standard Fenofibrate

Effect on Normocholesteremic Rats

The hyperlipidemic effects of the extracts were evaluated in 4 groups of Normocholesteremic rats fasted for 18hour and these studies were carried out as described for antihyperlipidemic effects the rats were treated orally for 7 days with the divided dose of 150 and 300mg/kg extracts p.o. After the end of the stipulated period of drug treatment, all animals were starved for 20hour and blood samples were collected from the puncture of retro-orbital plexus and analysed for blood lipid profile.

Group 1: Control

Group 2: 150 mg extract

Group 3: 300 mg extract

Group 4: Standard Fenofibrate

Biochemical Analysis of Serum

Serum samples were analysed for total cholesterol, High density lipoproteins, Low density lipoproteins and very low density lipoproteins using standard enzymatic assay kit.

Statistical analysis

Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Dunnnet t test using Graph Pad prism software package 9.05. Results were expressed as mean ± SD from 8 rats in each group. P values <0.05 were considered as significant.

6. RESULTS AND DISCUSSION

A) Extract values

Appearance and percentage yield of EECH(Ethanolic Extract of Alstonia scholaris leaves).

Methanolic extract of *Alstonia scholaris* was semisolid brownish colour extract and the percentage yield was found to be 16.38%

B) Screening of Phytochemical aspects of *Alstonia Scholaris*

S.No	Parameters	Aqueous portion	Ethyl acetate portion	Butanol portion
1	**Alkaloids**			
	Dragendroff reagent	+	-	-
	Wagner's reagent	-	-	-
	Mayer's reagent	-	-	-
2	**Flavonoids**	+	+	+
3	**Tannin**	+++	+	++
4	**Protein**	+++	+	++
5	**Saponin**	+	+	+
6	**Glycosides**	+	+	+
7	**Phenols**	+++	+	++
8	**Thiols**	-	-	-
9	**Steroids**	+++	+	++
10	**Carobohydrates**	++	+	+

Table 3. Screening of Phytochemical aspects of *Alstonia Scholaris*

+++ : Abundantly

++ : Moderately

\+ : Slightly

_ : Absence

Based on the preliminary phytochemical evaluation the following phytochemical constituents tannin ,protein, phenols and steroids abundantly presents, carbohydrates are moderately presents and flavonoids, saponin and glycosides are slightly presents in *Alstonia Scholaris*.above said phytochemical constituents may possess the pharmacological activity.

C) Acute Toxicity

Dose Determination

During preliminary toxicity study, no adverse effects or mortality was observed in experimental animals with oral administration of Stem extract up to a high dose of 5 gm/kg body weight observed for 24 hrs. Hence submaximal doses of 150 mg/kg and 300 mg/kg were selected as a test dose.

The toxicity for the aqueous and ethanolic extracts stem of *Alstonia scholaris* was determined in albino mice, maintained under standard conditions. The animals were fasted overnight prior to the experiment. Fixed dose (OECD) Guideline No. 423) method of CPSEA was adopted for toxicity studies. There were no sign of toxicity for first 48 hours and no animal died on 14 day of study at a dose of 2000 mg/kg.

D) ANTI OXIDANT STUDIES

DPPH Assay

Conc. (µg/ml)	Ascorbic acid	Extract 150 mg	Extract 300mg
25	67.34±0.77	62.45±0.76	67.65±0.60
50	71.56±0.12	63.67±0.88	69.73±0.70
75	77.43±0.41	65.80±0.31	73.73±0.09
100	79.12±0.20	68.69±0.90	78.08±0.61
150	88.43±0.72	75.90±1.40	86.04±0.63
200	93.07±0.70	77.04±0.55	89.87±0.43

Table 4. DPPH Assay

**Ascorbic acid, extracts of leaves (150mg/kg & 300mg/kg) % inhibition

All the experiments were performed in triplicates

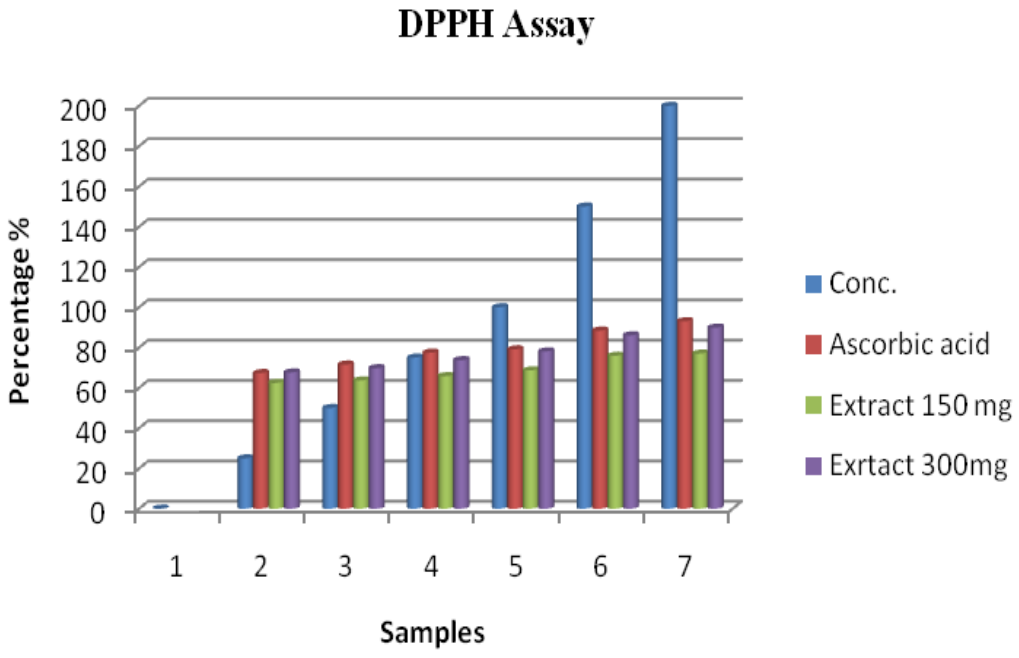

Fig 15. Schematic representation of DPPH activity of all the extracts

There has been an significant inhibition of free radicals has been observed with the both the 150mg/kg & 300mg/kg extract as compared with the standard ascorbic acid with the concentrations of 25, 50, 75, 100, 150 & 200 µg/ml respectively. There has been an considerable inhibition of the formed free radicals with the constituents present in both the samples.

Lipid per oxidation Assay

Conc. (µg/ml)	Ascorbic acid	Extract 150 mg	Exrtact 300mg
25	70.12±1.98	46.59±1.26	55.90±0.51
50	75.36±0.39	54.60±55	62.54±0.84
75	81.22±1.42	62.73±0.63	70.53±0.07
100	84.62±0.40	67.32±0.12	75.40±0.42
150	88.42±0.74	72.93±1.04	84.60±0.38
200	90.73±0.61	79.76±0.42	87.50±0.73

Table 5. Lipid per oxidation Assay

**Ascorbic acid, extracts of leaves (150mg/kg & 300mg/kg) % inhibition

All the experiments were performed in triplicates

There has been an significant inhibition of free radicals has been observed with the both the 150mg/kg & 300mg/kg extract as compared with the standard ascorbic acid with the concentrations of 25, 50, 75, 100, 150 & 200 µg/ml respectively. There has been a considerable inhibition of the formed free radicals with the constituents present in both the sampl

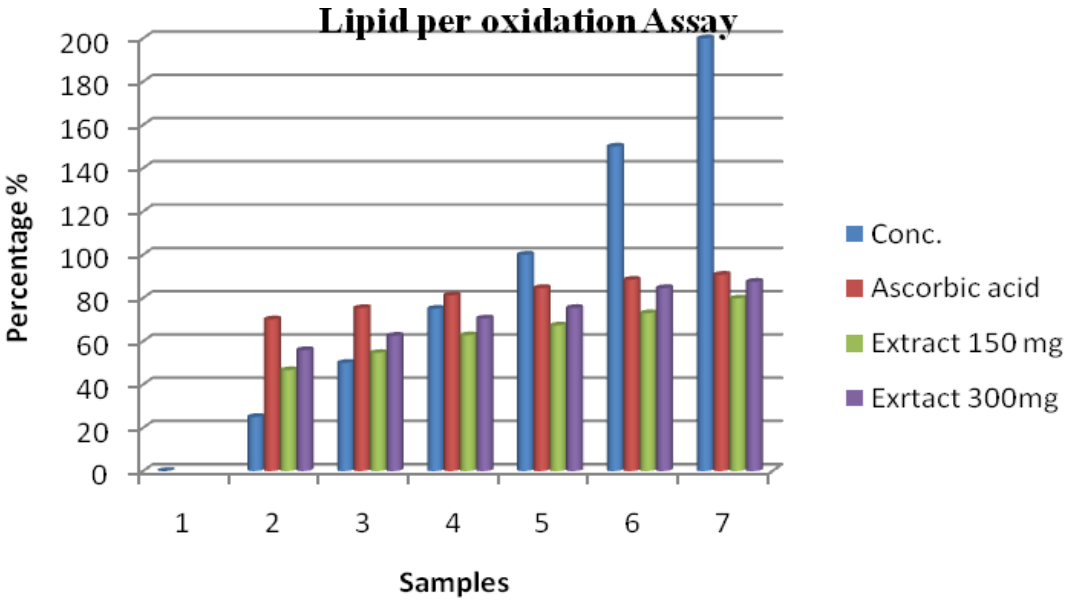

Fig 16. Schematic representation of Lipid per oxidation Assay of all the extracts

Nitric oxide scavenging assay

Conc. (µg/ml)	Ascorbic acid	Extract 150 mg	Exrtact 300mg
25	45.56±0.66	31.76±0.84	36.44±1.54
50	56.87±0.64	37.54±0.87	50.73±0.34
75	69.54±0.33	43.70±0.87	61.59±0.80
100	81.62±0.45	51.04±0.43	73.53±0.90
150	95.43±0.65	61.09±0.43	88.43±0.86
200	96.70±1.65	70.37±0.57	94.40±0.53

Table 6. Nitric oxide scavenging assay

**Ascorbic acid, extracts of leaves (150mg/kg & 300mg/kg) % inhibition. All the experiments were performed in triplicates

There has been an significant inhibition of free radicals has been observed with the both the 150mg/kg & 300mg/kg extract as compared with the standard ascorbic acid with the concentrations of 25, 50, 75, 100, 150 & 200 µg/ml respectively. There has been an

considerable inhibition of the formed free radicals with the constituents present in both the samples.

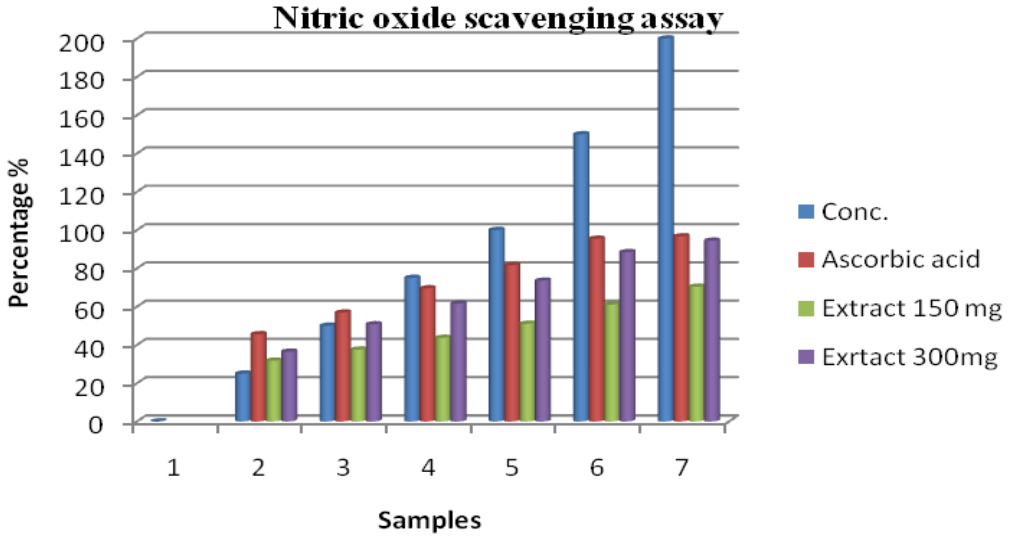

Fig 17. Schematic representation of Nitric oxide scavenging assay of all the extra

Hydrogen per oxide assay

Conc. (µg/ml)	Ascorbic acid	Extract 150 mg	Exrtact 300mg
25	49.76±0.33	39.78±0.36	46.19±0.56
50	59.65±0.73	47.65±0.09	54.63±0.87
75	68.65±0.43	55.73±0.93	63.76±0.74
100	74.67±0.84	61.56±0.54	70.39±0.73
150	83.78±0.60	70.41±0.97	78.65±1.33
200	86.12±0.54	76.00±0.86	87.33±0.54

Table 7. Hydrogen per oxide assay

**Ascorbic acid, extracts of leaves (150mg/kg & 300mg/kg) in % inhibition

All the experiments were performed in triplicates

There has been an significant inhibition of free radicals has been observed with the both the 150mg/kg & 300mg/kg extract as compared with the standard ascorbic acid with the concentrations of 25, 50, 75, 100, 150 & 200 µg/ml respectively. There has been an considerable inhibition of the formed free radicals with the constituents present in both the samples.

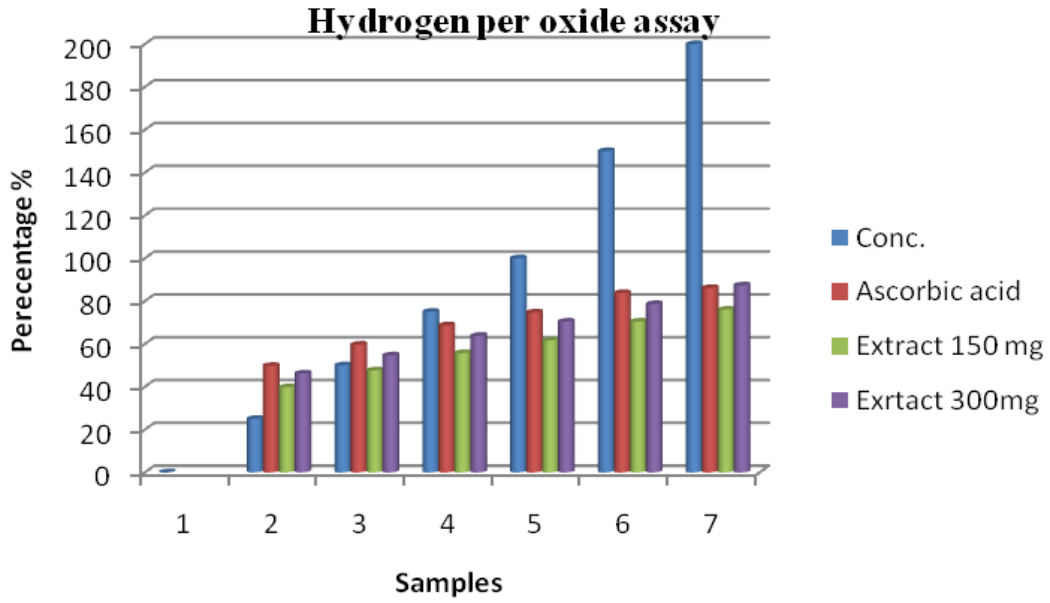

Fig 18. Schematic representation of Hydrogen peroxide assay of all the extracts

E) HIGH-CHOLESTEROL DIET INDUCED RAT MODEL.

Group 1: Animals were fed with a standard diet and was given 0.9% saline once daily for 8 weeks with the aid of oropharyngeal cannula.

Groups 2: Animals served as hypercholesterolemic (fed with 2% w/w pure cholesterol enriched diet) negative control.

The animals in group 3, 4 and 5 were fed with 2% w/w pure cholesterol enriched diet supplemented orally with 1 ml of the extract corresponding to 150, 300 mg and standard mg/kg per bwt (LD_{50}>2000), respectively, once daily for 8 weeks.

Sample	Treatment (mg/kg/b.wt)				
Serum	**Control**	**HC**	**HC+ 150 mg**	**HC+ 300mg**	**Standard** (FB)
TC	11.52±0.56	23.67±0.26	14.76±0.23	12.45±0.94	10.56±0.44
TG	7.36±0.56	6.02±67	5.56±0.77	5.02±0.45	4.02±0.66
LDL	3.98±0.23	8.56±0.56	3.78±0.67	3.42±0.60	3.23±0.43
HDL	4.91±0.75	2.96±0.04	2.22±0.54	2.45±0.67	2.87±0.22

HC- High Cholesterol; FB-Fenofibrate; TC-total cholesterol; TG- triacylglycerol;

LDL- low density lipoprotein; HDL- High density lipoprotein

Table 8. Cholesterol induced diet model

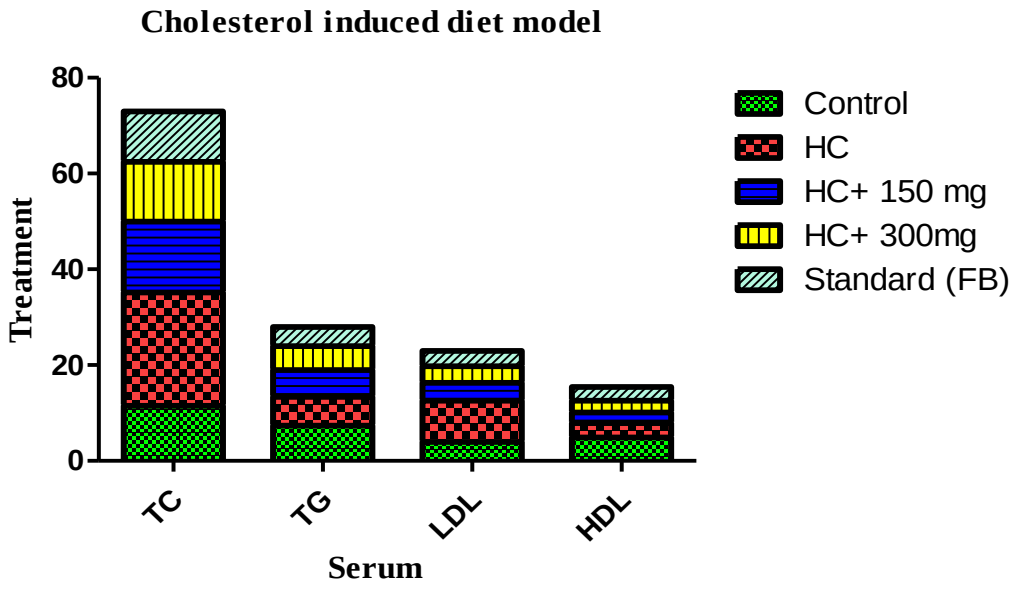

Fig 19. Cholesterol induced diet model

Study of cholesterol and phospholipids in serum and liver tissue of control and experimental animal

Groups	Cholesterol		Phospholipids	
	Serum	Liver	Serum	Liver
Control	87.0±0.76	62.0±0.76	51.0±0.56	86.0±0.72
150mg/kg+Triton	100.0±0.78	99.0±0.56	66.3±0.74	94.0±0.65
300 mg/kg+Triton	86.4±0.67	68.5±0.67	57.0±0.45	92.0±0.54
Standard Fenofibrate	72.7±0.55	65.7±0.88	50±0.54	85±0.54

Each value is mean ± SD for eight rats in each group, one way ANOVA followed by Dunnet t test.

Table 9: Effect of *Alstonia scholaris* on changes in the levels of cholesterol and phospholipids in serum and liver tissue of control and experimental animal

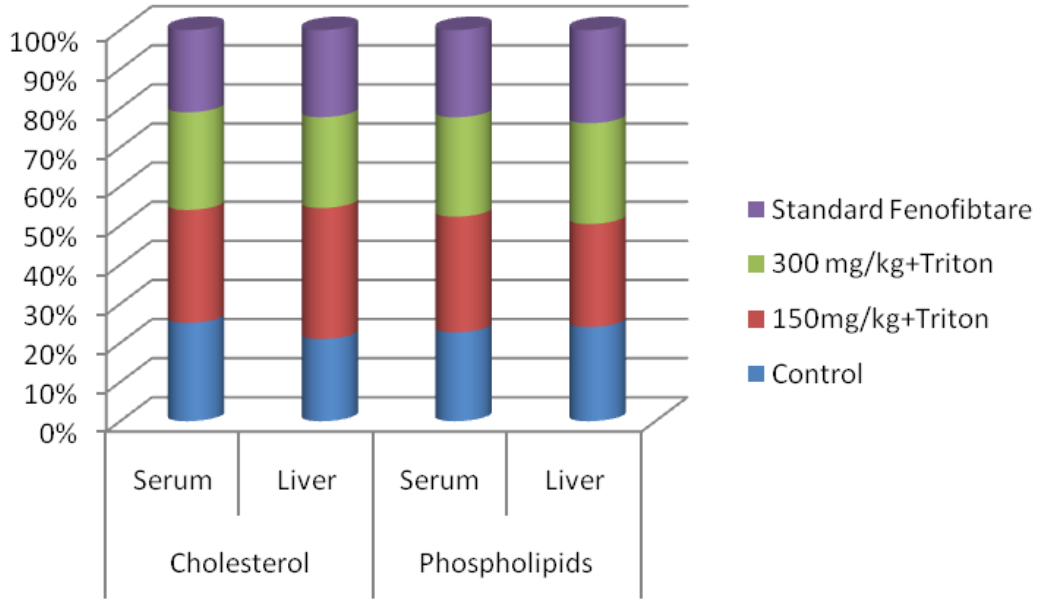

Fig 20. Scheme of Effects of Cholesterol and Phospholipids

Study of cholesterol and phospholipids in serum and liver tissue of triglycerides and LDL control and experimental animal

Groups	Triglycerides		LDL	
	Serum	Liver	Serum	Liver
Control	76.3±0.44	62.5±0.60	39.5±0.67	22.7±0.78
150mg/kg+Triton	97.5±0.54	94.9±0.43	67.5±0.63	36.3±0.72
300 mg/kg+Triton	89.2±0.64	67.4±0.54	63.5±0.90	32.5±0.43
Standard Fenofibrate	70.8±0.08	60±0.64	37.4±`1.6	22.3±0.75

Each value is mean ± SD for eight rats in each group, one way ANOVA followed by Dunnet t test.

Table 10: Effect of Alstonia scholaris on changes in the levels of triglycerides and LDL in serum and liver tissue of control and experimental animal

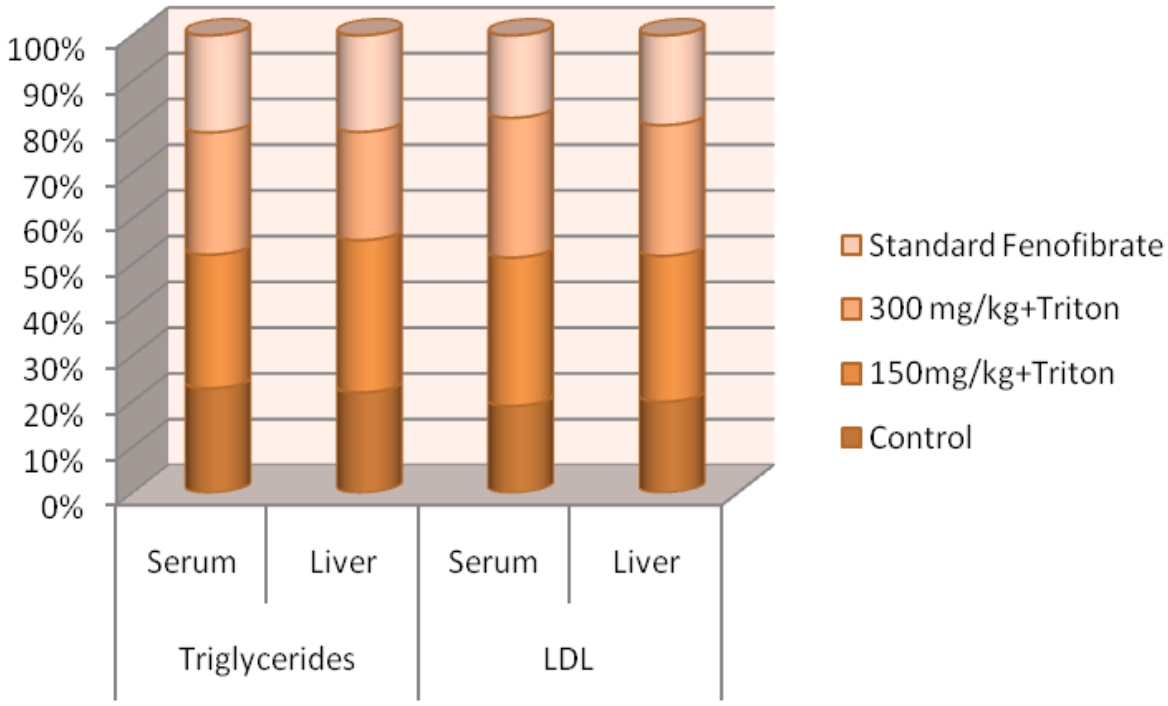

Fig 21. Scheme of Effects of triglycerides and LDL

Study of cholesterol and phospholipids in serum and liver tissue of triglycerides and LDL control and experimental animal

Groups	VLDL		HDL	
	Serum	Liver	Serum	Liver
Control	17.2±1.54	13.6±1.67	50.5±0.86	31.5±0.77
150mg/kg+Triton	16.4±0.83	15.4±0.83	46.5±0.97	27.5±0.87
300 mg/kg+Triton	15.4±0.95	12.3±0.56	37.4±0.72	24±0.61
Standard Fenofibrate	13.51±0.26	11.5±0.54	29.3±0.56	18±0.76

Each value is mean ± SD for eight rats in each group, one way ANOVA followed by Dunnet t test.

Table 11: Effect of Alstonia scholaris on changes in the levels of VLDL and HDL in serum and liver tissue of control and experimental animal

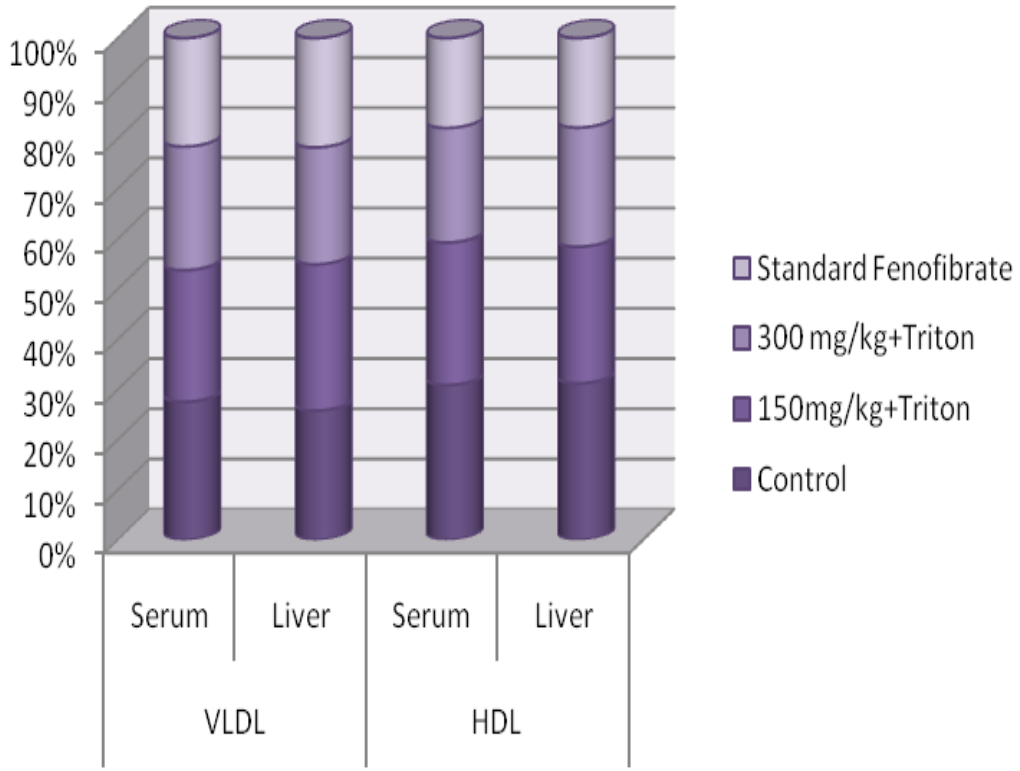

Fig 22. Scheme of Effects of VLDL and HDL

EFFECT ON NORMOCHOLESTEREMIC RATS

The hypolipidemic effects of the extracts were evaluated in 4 groups fasted for 18 hours and these studies were carried out as described for antihyperlipidemic effects. The rats were treated orally for 7 days. After the end of the stipulated period of drug treatment, all the animals were starved for 20 hours and blood samples were collected from the puncture of retro-orbital plexus and analyzed for blood lipid profile.

Groups	Blood lipid profile			
	Cholesterol	Triglycerides	HDL	LDL
Control	76.23±0.37	64.52±0.52	27.59±0.12	62.33±0.71
150 mg extract	72.31±0.78	59.99±0.51	31.68±0.77	51.76±0.16
300 mg extract	66.33±0.28	55.67±0.62	34.29±0.67	42.98±0.72
Standard Fenofibrate	64.15±0.91	51.22±0.39	33.26±0.58	40.33±0.17

Table 12. Effect of the extracts on blood lipid profile

In statistical analysis the extract treated groups have been compared with their respective control. P<0.01 (ANOVA followed by Dunnett's t-test)

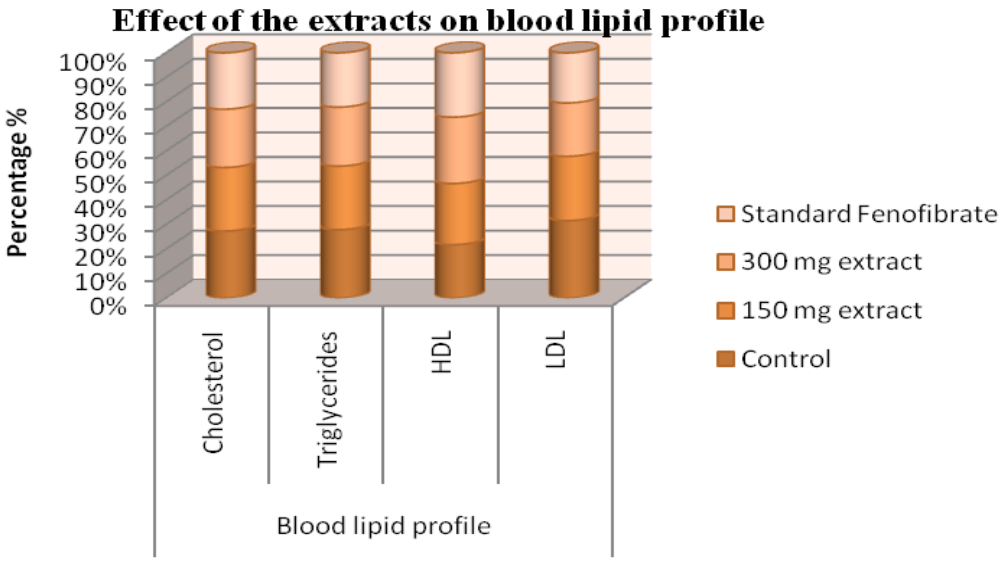

Fig 23. Scheme of Blood lipid profiles

The Antihyperlipidemic and antioxidant activity of the plant stem extract is studied and the significance is evaluated.

7. SUMMARY

In this study the leaf extract of ***Alstonia Scholaris*** has been utilized to assess the antioxidant activity and antihyperlipidemic activity, as the proposed plant leaves has been subjected to various studies and the results are complied in the previous sections. All these results are significantly remarkable and noteworthy.

Photochemical screening of the leaf extracts shows that the presence of primary components responsible for the antihyperlipidemic activity. Furthermore it also confirms the presence of polyphenolic moieties which may be responsible for the antioxidant activities also. The presence of the moieties belonging to flavonoids, alkaloids, tannins, proteins, carbohydrates, saponins, glycosides and steroids are conformed.

The acute toxicity studies shows that the even with 5 grams/kg body weight of animals does not show and significant adverse effect of the selected animal model. High cholesterol diet model has been studied. Very significant reduction in the cholesterol has been observed with the extract of 300mg/kg as compared with the standard drug.

There has been an significant inhibition of free radicals in case of DPPH has been observed with the both the 150mg/kg & 300mg/kg extract as compared with the standard ascorbic acid with the concentrations of 25, 50, 75, 100, 150 & 200 µg/ml respectively. There has been an considerable inhibition of the formed free radicals with the constituents present in both the samples. There has been an significant inhibition of free radicals in case of lipid peroxidation has been observed with the both the 150mg/kg & 300mg/kg extract as compared with the standard ascorbic acid with the concentrations of 25, 50, 75, 100, 150 & 200 µg/ml respectively. There has been an considerable inhibition of the formed free radicals with the constituents present in both the samples. There has been an significant inhibition of free radicals in case of Nitric oxide scavenging has been observed with the both the 150mg/kg & 300mg/kg extract as compared with the standard ascorbic acid with the concentrations of 25, 50, 75, 100, 150 & 200 µg/ml respectively. There has been an considerable inhibition of the formed free radicals with the constituents present in both the samples.

There has been an significant inhibition of free radicals in case of Hydrogen peroxide has been observed with the both the 150mg/kg & 300mg/kg extract as compared with the standard ascorbic acid with the concentrations of 25, 50, 75, 100, 150 & 200 µg/ml

respectively. There has been an considerable inhibition of the formed free radicals with the constituents present in both the samples.

The triton induced hyperlipidemia (Cholesterol & Phospholipids) has also been studied the results shows that the 300mg/kg body weight with Triton has comparatively good as compared with that of control group, and further relatively better controlled the hyperlipidemia as compared with that of the standard drug in both serum and liver concentration. The triton induced hyperlipidemia (Triglycerides & LDL level) has also been studied the results shows that the 300mg/kg body weight with Triton has comparatively good as compared with that of control group, and further relatively better controlled the hyperlipidemia as compared with that of the standard drug. The triton induced hyperlipidemia (VLDL & HDL level) has also been studied the results shows that the 300mg/kg body weight with Triton has comparatively good as compared with that of control group, and further relatively better controlled the hyperlipidemia as compared with that of the standard drug.

The effect of both the concentrations of the extract shows that the 300mg/kg extract shown comparatively and resembles the lipid controlling of the standard drug, in addition to that the control group has little less control of lipid profile as compared with both the concentrations of the extracts.

8.CONCLUSION

In this study, the leaf methanolic extract of ***Alstonia scholaris*** used for the determination of antihyperlipidemic and antioxidant activity, acute toxicity studies also done which shows no adverse effect even at the higher concentration of the drug. The phyochemical screening shows the essential useful component for the formulation. The obtained leaf extract at higher concentration have the better antihyperlipidemic and antioxidant property as compared with the standard drug. Hence the leaves of *Alstonia Scholaris* has the potential for the above mentioned properties.

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